PATHOLOGICAL AND PHYSIOLOGICAL STUDIES OF SOYBEAN SEED QUALITY

By

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Dedicated to Andrea, Marcelo, and

Luciana, with love and hopes

for a bright future.

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PATHOLOGICAL AND PHYSIOLOGICAL STUDIES OF SOYBEAN SEED QUALITY

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Pathological and physiological qualities of soybean [Glycine max (L.) Merrill] seeds were studied. In a survey of the quality of commercial soybean seeds produced in Florida in 1986, mechanical damage was the most detrimental factor affecting seed quality, followed by weathering and stink bug damage. Due to climatic conditions, seed produced in the eastern region of the Florida panhandle had lower levels of infection by several fungi. Apparently this region is more suitable for production of soybean seed.

Phomopsis spp. were the fungi most frequently associated with soybean seeds (up to 77% incidence). Seed infection was mainly restricted to the seedcoat. Rolled paper toweling was not the best substrate to evaluate germination of soybean seed infected with high incidence of Phomopsis spp. Emergence in sand, which simulates emergence in soil,

provided a more representative estimate of viability. The tetrazolium test provided similar results to the emergence-in-sand test.

Seed infection by Colletotrichum truncatum (Schw.) Andrus and Moore was mainly restricted to the seedcoat, but 15% of the seed samples had up to 10% embryo infection. Although C. truncatum infected fewer seeds than Phomopsis spp., it caused more damping-off. Emergence in sand provided better estimates of viability for seeds infected by C. truncatum than the standard germination test.

Cercospora kikuchii (Matsu. and Tomoyasu) Gardner was almost exclusively restricted to the seedcoat. An antagonistic effect between C. kikuchii and Phomopsis spp. was observed. No detrimental effects of seed infection by C. kikuchii were observed on germination, emergence, and vigor of soybean seeds.

The tetrazolium test can be used for estimating germination and vigor of soybean seeds. The test also diagnoses the causes that contribute to lower seed quality, such as mechanical, weathering, and stink bug damages. The tetrazolium test will enable seed producers to identify and correct the causes that affect soybean seed quality. The test is not influenced by the presence of fungi infecting soybean seed. Therefore, this technique can help the researcher visualize and understand some processes of seed deterioration. When combined with bio-assays, the test allows the researcher to separate fungal deterioration from other sources of deterioration.

CHAPTER I
INTRODUCTION

The major soybean [Glycine max (L.) Merrill] seed and grain production areas were restricted to temperate regions of the world until the mid 1940s. After that period, and especially during the last decade, the area of production has spread into subtropical and tropical regions. These regions present warmer and more humid conditions, and as a result, seed growers have experienced increased difficulties in consistently producing soybean seed of high viability and quality.

The successful production of good yields of soybean strongly depends upon the use of good quality seed. The use by the soybean grower of seeds with lower vigor might result in poor stands and reductions in yield. In some instances replanting will be necessary, and important losses are associated with this practice.

Seed deterioration is a natural process that involves cytological, physiological, biochemical, and physical changes in each seed which eventually causes the seed to die. It has been characterized as progressive, irreversible, and inexorable (Delouche, 1973). Seed deterioration is determined by genetic factors, preharvest environmental conditions, harvesting and processing procedures, and environment during storage and transit.

There are several factors that contribute to lowering the quality of soybean seed. The major ones, as described by Franca Neto (1984) and Moore (1960, 1962a, 1973), are mechanical and weathering damages, stink bug damage, heat and drought damage, and freeze injury. Several pathogens also affect soybean seed quality. Phomopsis spp., Colletotrichum truncatum, Cercospora kikuchii, and Fusarium spp. are among the fungi most frequently associated with soybean seeds (Henning, 1985). In spite of being distinct factors, the action and interaction of all these physiological, physical, and pathological factors contributed to a common result: seed deterioration.

Several of the above factors were studied and reported herein. The studies reported in Chapter II were conducted with the objectives of: a) to evaluate the physiological and pathological qualities of commercial soybean seeds produced in Florida; and b) to diagnose the major factors that decrease soybean seed quality and recommend practices that might result in improvement of quality of soybean seeds produced in Florida.

Chapter III reports on research conducted with the objectives of a) to determine the influence of Phomopsis spp. in evaluating germination of soybean seeds by the standard germination, emergence-in-sand, and tetrazolium tests; and b) to recommend alternative methods that correctly evaluate germination of soybean seed lots infected with Phomopsis spp.

The objectives of the study reported in Chapter IV were: a) to determine the influence of Colletotrichum truncatum on evaluating the viability of soybean seeds by the standard germination, emergence in sand, and tetrazolium tests; b) to determine the best method to evaluate

germination of seeds infected with the pathogen; and c) to investigate the depth of infection by this pathogen in soybean seeds.

The main objective of the study reported in Chapter V was to study the effects of *Cercospora kikuchii* on the quality of soybean seeds produced in Florida. In addition, a discussion about possible reasons for some inconsistent conclusions about seed infection of soybean seeds by *C. kikuchii* as reported in the literature will be presented.

Several features of tetrazolium testing of soybean seeds are considered in Chapter VI: a) the major events and accomplishments which contributed to the development and perfecting of the test; b) the basic principles of the test; c) needed equipment and supplies; d) procedures for seed preparation and evaluation; e) basis for the correct interpretation of the results; f) advantages and limitations of the test; and g) accuracy of the results. In addition, some research data obtained from soybean seed lots produced in Florida will be presented.

CHAPTER II
MULTIPLE QUALITY EVALUATION OF SOYBEAN SEED PRODUCED IN FLORIDA IN 1986

It is estimated that approximately 20% of all soybean seed planted in Florida are either certified or commercial seed produced within the state (W. R. Vaughan, 1987, personal communication). The remaining 80% are either imported from other states or are produced by a few farmers who save their own seed. These statistics clearly reflect how difficult it is to produce high-quality soybean seed in Florida.

Several factors can affect soybean seed quality. Among the most important are field deterioration, mechanical damage, stink bug damage, and infection by several fungi (Franca Neto and Henning, 1984). Field deterioration or weather damage occurs between physiological maturity and harvest. Exposure of soybean seed to alternating wet and dry conditions associated with high temperatures causes seedcoat wrinkling (Moore, 1973; Wolf et al., 1981; Pereira and Andrews, 1985), a typical symptom of field deterioration. Embryonic tissues just beneath the seedcoat become damaged during wrinkling, which may result in reduced seed vigor and a higher level of abnormal seedlings (Moore, 1972; Pereira, 1974). In addition, field deterioration can predispose soybean seed to more mechanical damage at harvest (Delouche, 1972; Franca Neto et al., 1984).

The soybean seedcoat is very thin and low in lignin content, and provides little protection to the fragile radicle which lies in a vulnerable position directly beneath the seedcoat (Agrawal and Menon, 1974; Gupta et al., 1973; Franca Neto and Henning, 1984). Limited protection is provided by the cushioning effect by the hourglass cells (Pereira and Andrews, 1985), and by a seedcoat-derived pocket that surrounds the radicle tip (McDonald et al., 1987). Soybean seed should be harvested as soon as possible after the seed moisture content reaches 15 to 12% (Matthes, 1971; Delouche, 1972; Costa et al., 1979). Seed cracking and splitting increase when moisture content decreases below 12%, and seed bruising and other less visible injuries increase at moisture contents above 15% (Matthes, 1971; Delouche, 1972). Both types of damage can be easily recognized by the tetrazolium test (Franca Neto et al., 1985b).

Stink bugs can seriously reduce soybean seed quality. Several species attack soybeans in the United States. The green stink bug, _Acrosternum hilare_ (Say) and the southern green stink bug _Nezara viridula_ (L.), are the most important (Turnipseed and Kogan, 1976). The green stink bug occurs throughout all soybean growing areas in the United States, but _N. viridula_ is limited to southern U.S. (Yeargan, 1977). When stink bugs feed on seed they also inoculate them with the yeast fungus _Nematospora coryli_ Peglion (Sinclair, 1982). Colonization of this fungus in seed tissue often results in severe losses of seed viability and vigor and grain quality (Bowling, 1980; Villas Boas et al., 1982). This infection results in characteristic lesions that can

be readily identified by the tetrazolium test (Franca Neto et al., 1985b).

Several pathogens also affect soybean seed quality. Because of their greater number and resulting losses, fungi are considered most important (Franca Neto and Henning, 1984). The level of fungal infection increases if seed mature in humid and warm environments or if harvest is delayed (Wilcox et al., 1974; Nedrow and Harman, 1980; Sinclair, 1982; Henning and Franca Neto, 1985; Hill et al., 1985). *Phomopsis* spp., *Colletotrichum truncatum*, *Cercospora kikuchii*, and *Fusarium* spp. are among the fungi most frequently associated with soybean seeds (Henning, 1985). Several researchers consider *Phomopsis* spp. to be the most important fungus associated with soybean seed, and several studies have been carried out to understand this pathogen better (Kulik, 1985).

Quality evaluations (Zappia et al., 1980; Costa et al., 1982, 1985a, 1985b, 1986, 1987) of soybean seed produced in several regions have provided valuable information to the soybean seed industry. These surveys indicate possible flaws in the production system, identify cultivars best suited for specific regions, and suggest areas best suited for seed production. Studies performed in the state of Parana, Brazil (Costa et al., 1982, 1985b, 1986, 1987) is an outstanding example of quality evaluation.

The objectives of the study reported here were to: a) evaluate the physiological and pathological qualities of commercial soybean seed produced in Florida in 1986; and b) diagnose the major factors that decrease soybean seed quality and recommend practices that might result in improvement of quality of soybean seed produced in Florida.

Materials and Methods

Forty-three samples of commercial soybean seed produced in Florida in 1986 were obtained from the State Seed Laboratory in Tallahassee. These samples represented approximately 22% of the total commercial soybean seed produced in the state (W. R. Vaughan, 1987, personal communication).

All seed were produced in two ecological regions of Northwest Florida designated A and B (Fig. 1). Region A is known to receive more rainfall than region B during October and November, when soybeans mature and are harvested (Dr. E. B. Whitty, 1987, personal communication). This trend occurred in 29 out of 40 years during the period from 1948 to 1987 (compiled from National Oceanic and Atmospheric Administration, 1948 to 1987).

The 43 samples represented eight soybean cultivars (Table 1). The highest number of seed lots was of 'Centennial', followed by 'Kirby' and 'Gordon'. These cultivars represented 74.4% of all the samples analyzed. The following tests were performed on the samples.

Standard germination was performed according to procedures outlined for soybeans in the Rules for Testing Seeds (Association of Official Seed Analysts - AOSA, 1981). Four replications of 50 seed each were placed in rolled paper toweling and stored at 25°C for five days.

Emergence in sand was determined for two replicates of 100 seeds each. Each replicate was planted in a 10 X 23 X 30cm plastic tray containing 3,00 mL of washed, air-dried sand. Seed were planted 3.0 cm deep, and 450 mL of deionized water were added to each tray just after

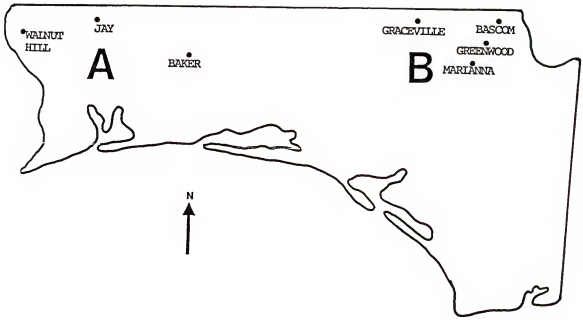


Fig. 1. Soybean producing areas in the panhandle of the state of Florida: region A, which encompasses Walnut Hill, Jay, and Baker, and region B, composed of Graceville, Bascom, Greenwood, and Marianna.

Table 1. Number of lots tested at the Agronomy Seed Laboratory of the University of Florida for the quality evaluation of soybean seeds produced in Florida in 1986.

Cultivar	Number of lots			%
	Region A[a]	Region B[b]	Total	
Centennial	14	5	19	44.2
Kirby	5	2	7	16.3
Gordon	5	1	6	13.9
Braxton	0	4	4	9.3
Leflore	2	1	3	7.0
Jones	2	0	2	4.7
Gasoy	0	1	1	2.3
Coker 488	0	1	1	2.3
TOTAL	28	15	43	100.0

[a]Region A : Jay, Walnut Hill, and Baker.
[b]Region B: Bascom, Marianna, Graceville, and Greenwood.

planting. Approximately 100 mL of water were added to each tray every other day to keep the sand moist. Trays were kept at 25°C, and readings were made on the 5th and 10th day after planting. Evaluation of seedlings was performed according to the Rules for Testing Seeds (AOSA, 1981).

Tetrazolium (tz) tests were performed according to procedures described by Delouche et al. (1962), and Moore (1973), and modified by Franca Neto et al. (1985b). Two replicates of 50 seeds each were used per sample. Seeds were preconditioned in moist paper toweling overnight for 16 hours at 25°C. The seeds were then placed in 50-mL plastic cups and covered with 0.075% solution of 2,3,5 triphenyl tetrazolium chloride, and incubated at 40°C for 2.5 hours. The seeds were rinsed thoroughly with cool, running tap water, and left immersed in water until evaluations were made. Seeds were evaluated for germination, vigor potential, and levels of mechanical damage, field deterioration (weathering), and stink bug damage. See Chapter VII for more detailed information about the tz test.

Blotter test was used to determine the percentage of infected seeds. Seeds were surface sterilized for one minute in a 1.05% solution of sodium hypochlorite, and thoroughly rinsed in deionized water. Seeds were placed on two layers of moist germination paper in 14.0 cm diameter Petri dishes. Five dishes containing 20 seeds each were used per sample. Seeds were incubated for seven days at 25°C under eight hours of light (daylight fluorescent) before the determinations of infected seeds were performed.

Rainfall and temperature (maximum and minimum) data collected at Jay and Marianna represent regions A and B, respectively. These data were kindly provided by Dr. Darell McCloud and Mr. Richard Hill, Agronomy Department, University of Florida.

This experiment was not planned to be analyzed statistically due to the sampling procedures.

Results and Discussion

Rainfall at Jay (region A) during October and November, 1986, was much higher than it was at Marianna (region B) (Fig. 2), which is consistent with the general trend that region A receives more rainfall than region B during this period (National Oceanic and Atmospheric Administration, 1948 to 1987). Temperatures recorded for both regions were similar, with mean values near 21°C and 16°C for October and November, respectively.

Based on rainfall differences for October and November (Fig. 2) one can infer that region B should be better suited for soybean seed production. The lower incidence of *Phomopsis* spp., *Fusarium* spp., *Colletotrichum truncatum*, and *Cercospora kikuchii* in seeds from region B (Table 2) support this inference. For all 43 seed lots, *Phomopsis* spp. infection was 21.7% in region A but only 5.1% in region B. Effects of *Phomopsis* spp., *Fusarium* spp., and *C. truncatum* on seed viability were of particular interest, since Franca Neto et al. (1983) reported that *C. kikuchii* did not cause any significant decrease in seed germination and emergence in Brazil.

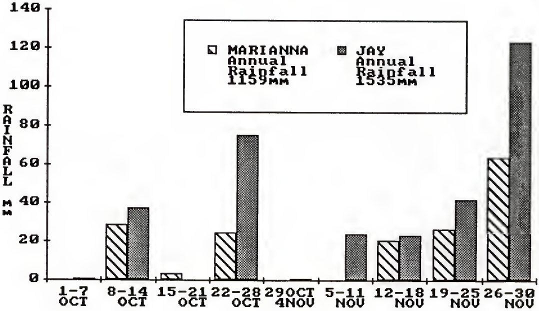


Fig. 2. Weekly precipitation for Jay, representing region A, and Marianna, representing region B, during October and November 1986.

Table 2. Mean levels of *Phomopsis* spp., *Fusarium* spp., *Colletotrichum truncatum*, and *Cercospora kikuchii* as determined by the blotter test performed on soybean seed lots produced in Florida in 1986.

Cultivar	Region	*Phomopsis* spp.	*Fusarium* spp.	*C. truncatum*	*C. kikuchii*
		%			
Centennial	A	30.3	9.4	3.1	2.1
	B	5.2	4.8	0.0	1.2
	Mean[a]	23.7	8.2	2.3	1.9
Kirby	A	15.4	10.8	1.8	3.2
	B	0.5	1.5	0.0	0.5
	Mean[a]	11.1	8.1	1.3	2.4
Gordon	A	13.4	17.6	7.2	9.2
	B	10.0	4.0	0.0	4.0
	Mean[a]	12.8	15.3	6.0	8.3
All 43 lots	A	21.7	11.8	4.9	3.8
	B	5.1	7.2	0.2	2.4
	Mean[a]	15.9	10.2	3.3	3.3

[a]Weighted mean.

Among the cultivars, Kirby had the lowest levels of infection by Phomopsis spp., Fusarium spp., and C. truncatum and the best viability (standard germination, emergence in sand, and tz germination) and vigor levels, as illustrated in Tables 2 and 3. Kirby normally matures two weeks later than Centennial and Gordon, and this usually coincides with less adverse climatic conditions regarding rainfall and temperature. In addition, the results in Table 3 illustrate that commercial soybean seeds produced in Florida in 1986 were of low to medium quality.

The results reported for standard germination (paper towel), emergence in sand, and tz germination (Table 3) are measures of the same parameter, i.e., viability. Therefore, each seed lot should receive the same value for all three parameters. This was not the case. Considering all 43 seed lots the standard germination test produced the lowest value, which was followed by emergence in sand, then tz-germination. According to several reports (Henning and Franca Neto, 1980, 1985; Franca Neto et al., 1985a), Phomopsis spp. can drastically reduce germination in laboratory tests (rolled paper toweling), but may not affect emergence in the field or in sand if the physiological quality of the seed is good. In Brazil, seed lots with reduced germination in laboratory (rolled paper toweling) due to high levels of Phomopsis spp. could have high vigor and viability as determined by the emergence in sand and tz tests (Henning and Franca Neto, 1980, 1985). One possible explanation for this is that infection occurring late in the season resulted in internal but not deep-seated seedcoat infection. In such cases, seeds tested in rolled paper toweling have close contact between the infected seedcoats and cotyledons which increases the level

Table 3. Means for the results obtained for the standard germination test, emergence in sand, and tetrazolium test (germination and vigor) according to cultivar and region of production of soybean seed lots in Florida.

Cultivar	Region	Standard germination	Emergence in sand	Tetrazolium germination	Tetrazolium vigor[a]
		%			
Centennial	A	56.6	68.4	74.4	54.4
	B	61.8	65.0	67.6	44.6
	Mean[b]	57.9	67.5	72.6	51.8
Kirby	A	69.8	72.6	77.0	60.0
	B	85.5	82.5	79.5	66.5
	Mean[b]	74.3	75.4	77.7	61.9
Gordon	A	60.0	60.6	72.6	58.2
	B	78.0	70.0	71.0	58.2
	Mean[b]	63.0	62.2	72.3	58.2
All 43 lots	A	59.3	65.8	74.2	56.0
	B	65.9	66.4	67.5	47.8
	Mean[b]	61.7	66.2	71.9	53.1

[a]Vigor by tetrazolium: high $\geq$ 70%; medium = 69% to 50%; low $\leq$ 49%.
[b]Weighted mean.

of dead seed and infected seedlings. However, when seeds are tested in sand or soil, seedlings leave the infected seedcoats in or on the soil upon emergence, and in this way escape much of the detrimental effects of infected seedcoats.

These facts can explain the differences observed in the results obtained by the standard germination test and emergence in sand, especially for the seed lots produced in region A, which showed high levels of infection by *Phomopsis* spp. As reported previously, the results obtained by emergence in sand and tz-germination should be very much the same. Nevertheless, tz-germination was higher (Table 3). This can probably be explained by the fact that the tz test, being a biochemical test, was not affected by the presence of fungi within the soybean seedcoat, thus resulting in the highest values (Franca Neto et al., 1985a). Emergence in sand was not affected by superficial infections of *Phomopsis* spp. (Henning and Franca Neto, 1985), but could have been affected by the presence of *C. truncatum* (Sinclair, 1982), which could cause seedling damping-off. Standard germination, which had the lowest values, could have been affected by the presence of both fungi.

Table 4 contains the mean levels of mechanical damage, weathering, and stink bug damage detected by the tetrazolium test. These data indicate the percentage loss of viability due to each kind of damage. Values equal to or above 7.0% indicate a serious problem (Franca Neto et al., 1985a). The highest percentage loss of viability for commercial soybean seeds produced in Florida in 1986 was due to mechanical damage. Weathering, the second most serious factor, was expected to be the

Table 4. Mean levels of mechanical damage, weathering, and stink bug damage as detected by the tetrazolium test performed on soybean seed lots produced in Florida in 1986.

Cultivar	Region	Mechanical damage[a]	Weathering[a]	Stink bug damage[a]
		%		
Centennial	A	15.1	7.2	7.9
	B	13.8	20.6	8.2
	Mean[a]	14.8	10.7	7.9
Kirby	A	10.4	7.6	9.6
	B	8.0	12.0	4.0
	Mean[b]	9.7	8.9	8.0
Gordon	A	9.6	17.2	5.0
	B	22.0	6.0	5.0
	Mean[b]	11.7	15.3	5.0
All 43 lots	A	13.1	10.2	8.2
	B	16.5	14.6	8.5
	Mean[b]	14.3	11.2	8.3

[a]Serious problems with mechanical damage, weathering, and stink bug are indicated by values above 7.0%

[b]Weighted mean.

number one factor, due to the very stressful climatic conditions that usually occur during soybean maturation in Florida. Weathering damage for all 43 seed lots was higher for region B than for region A. The soybean is a relatively recent crop in region B, and possibly timeliness of harvest for commercial seed purposes was not the best for that region. Stink bug damage, on the average, was above the critical level of 7.0%, and also considered to be a serious problem.

High levels of mechanical damage were also reported for the state of Parana, Brazil (Costa et al., 1986, 1987), in quality evaluations of soybean seeds. After the problem was identified, farmers were instructed on combine adjustments, through wide-scale extension training programs. In two years, levels of mechanical damage were drastically reduced to acceptable levels.

Stink bug and mechanical damages are easily controlled factors. Considering that there are simple technologies that, if adopted, could result in tremendous improvement in soybean seed quality, the producers of commercial soybean seed in Florida should be warned and receive training in the application of proper technology.

The minimum standard for germination for commercial soybean seed in Florida is 60% (Florida Department of Agriculture & Consumer Services, 1984). Table 5 illustrates that 41.9% of all the seed lots showed standard germination (rolled paper toweling) below the minimum level. Viability measured by emergence in sand would reduce the level of non-approved seed lots to 27.9%. These data suggest that the standard germination test (rolled paper toweling) is not correctly evaluating the viability of soybean seeds infected with <u>Phomopsis</u> spp., as was the case

Table 5. Percentages of soybean seed lots falling within three different levels for the standard germination, emergence in sand, and tetrazolium (germination) tests.

Parameter	Parameter level	Cultivar			All lots
		Centennial	Kirby	Gordon	
	%	----------------------%[a]----------------------			
Standard germination	≥80	10.5	57.1	16.7	18.6
	60 to 79	42.1	28.6	50.0	39.5
	<60	47.4	14.3	33.3	41.9
Emergence in sand	≥80	26.3	42.9	0.0	20.9
	60 to 79	47.4	42.9	83.3	51.2
	<60	26.3	14.2	16.7	27.9
Tetrazolium germination	≥80	36.8	42.9	16.7	30.2
	60 to 79	42.1	57.1	66.6	51.2
	<60	21.1	0.0	16.7	18.6

[a]Percentage of seed lots within each level.

in Brazil (Franca Neto et al., 1985a; Henning and Franca Neto, 1985), and in one study in the USA (Schoen, 1985).

Data contained in Table 6 provide a diagnosis for the quality problems that contributed to decrease the quality of soybean seed produced in Florida in 1986. Among all the 43 seed lots, 67.4%, 51.2%, and 39.5% of such lots showed serious problems with mechanical damage, weathering, and stink bug damage, respectively.

Conclusions

The quality of soybean seed produced in Florida is affected by several different types of damage, incidence of specific fungi, cultivar, and location of production. Mechanical damage during harvest was the most detrimental factor affecting quality of soybean seeds, followed by weathering and then stink bug damage. *Phomopsis* spp. and *Fusarium* spp. were the fungi most frequently associated with soybean seeds produced in Florida in 1986. Kirby, a late maturity cultivar, maintained the best seed quality when compared to Centennial and Gordon. Due to its climatic conditions, seed produced in region B (Bascom, Marianna, Graceville, and Greenwood) had lower levels of infection by several fungi. This region is more suitable than region A for soybean production in Florida.

Apparently, rolled paper toweling was not the best substrate to evaluate viability of soybean seed lots with high levels of *Phomopsis* spp.

Table 6. Problems detected by the tetrazolium test which contributed to decreased soybean seed viability (emergence in sand < 80%) in Florida in 1986.

Cultivar	Problem		
	Mechanical damage	Weathering	Stink bug damage
	%[a]		
Centennial	68.4	47.4	31.6
Kirby	57.1	42.9	42.9
Gordon	66.7	66.7	16.7
All 43 lots	67.4	51.2	39.5

[a]Percentage of seed lots showing serious levels of the respective problem.

Better crop management practices would improve the quality of commercial soybean seed produced in Florida. Better crop management includes more timely harvest, better adjustments of combine harvesters, better control of stink bugs, and selection of more appropriate regions for seed production.

CHAPTER III
PROBLEMS IN EVALUATING VIABILITY OF SOYBEAN SEED INFECTED WITH *Phomopsis* spp.

Pod and stem blight disease of soybean and Phomopsis seed decay are caused by a group of fungi, including the conidial states *Phomopsis sojae* Lehman and *Phomopsis longicolla* Hobbs, and the ascal state *Diaporthe phaseolorum* (Cooke and Ellis) Saccardo var. *sojae* (Lehman) Wehmeyer (Hobbs et al., 1985; Kmetz et al., 1978; Lehman, 1922), hereafter referred to as *Phomopsis* spp. in this dissertation. These fungi infect seed prior to harvest during or after maturation when weather is moist, especially during warm periods (Balducchi and McGee, 1987; McGee, 1983; Rupe and Ferriss, 1986; Shortt et al., 1981; TeKrony et al., 1983).

The negative effects of *Phomopsis* spp. on germination of soybean seeds are broadly recognized in the laboratory by the rolled-paper-towel method (Henning and Franca Neto, 1980, 1985; Kulik and Schoen, 1981; Loeffler et al., 1988; Schoen, 1985; TeKrony et al., 1984; Thomison et al., 1988), or on culture media (Bisht and Sinclair, 1985; Hepperly and Sinclair, 1981; Wilcox et al., 1985). Nevertheless, controversial results have been published on the emergence of infected seed in sand or soil. McGee et al. (1980), and Paschal II and Ellis (1978) reported that emergence was negatively correlated with the incidence of *Phomopsis* spp. Gleason and Ferriss (1985a, 1985b) reported a similar trend, but

added that losses induced by Phomopsis spp. were greatest in relatively dry soil. Henning and Franca Neto (1980, 1985), and Franca Neto et al. (1985a) observed that Phomopsis spp. did not affect emergence in soil or sand if the physiological quality of the seed was good and environmental conditions were adequate for fast emergence. This controversial subject was emphasized by Kulik and Schoen (1981), who reported that seed infection may or may not affect the quality and field performance of seed lots, depending on place of production.

Rolled paper toweling, as prescribed by the Rules for Testing Seeds (Association of Official Seed Analysts - AOSA, 1981) is the simplest and most widely used method for the official evaluation of germination of soybean seed lots. Based on these facts, the question addressed by this work was: does this procedure correctly evaluate the germination of soybean seed infected with Phomopsis spp.?

The objectives of this study were to: a) determine the influence of Phomopsis spp. in evaluating germination of soybean seed by the standard germination, emergence-in-sand, and tetrazolium tests; and b) recommend alternative methods that correctly evaluate germination of soybean seed lots infected with Phomopsis spp.

Material and Methods

Seventy-three soybean seed samples of 23 cultivars and five breeding lines produced in Florida in 1986 were evaluated for quality at the Agronomy Seed Laboratory of the University of Florida. The following tests were performed on the samples.

Standard germination (rolled paper toweling), emergence-in-sand, and tetrazolium (tz) tests were performed on the seed samples according to the procedures described in Chapter II. Percentage seedling damping-off was also recorded from the emergence-in-sand test.

Blotter tests were used to determine fungal infection. To determine depth of infection in the seed, samples were evaluated with and without seedcoats: a) seedcoats were carefully removed with the help of a razor blade after preconditioning the seed in moist paper toweling overnight for 16 h at 25°C, after which seed were immersed in deionized water for 3 h at the same temperature; b) seed with intact seedcoats were surface sterilized for 1 min in a 1.05% solution of sodium hypochlorite, and thoroughly rinsed in deionized water. Seed with or without seedcoats were placed on two layers of moist germination paper in 14.0-cm diameter Petri dishes. Five dishes containing 20 seed each were used per sample. Percentage of infection was recorded after incubation for 7 days at 25°C under 8 h of light (daylight fluorescent).

Several simple correlations and regression analyses were performed on two sets of data. The first one encompassed 50 seed samples, with emergence in sand higher than 70%. The exclusion of the remaining 23 samples of low vigor seed was done to obtain a group of samples that would represent a population composed of high- and medium-quality seed lots, that perhaps could be used for planting purposes. The second set of data was composed of all 73 seed samples.

Results and Discussion

The ranges and means for several seed quality parameters for the 50 samples with emergence in sand higher than 70% are given in Table 7. The ranges for emergence in sand (ES) and tz-germination (TZG) were much the same (70% to 99%). Nevertheless, the range for the standard germination (G) was much wider, from 36% to 96%. Infection by Phomopsis spp. ranged from 0% to 77%, while deep-seated infection, as determined on seed without seedcoats, was low. Deep-seated infection was detected on only 9 out of 50 seed samples, with a maximum of 7% infection, and a mean level of 0.5%. These data agree with previous reports (Henning and Franca Neto, 1980; Henning, 1988) that Phomopsis spp. is usually confined to the seedcoat, but it might also colonize embryo tissues (Kunwar et al., 1985; Sinclair, 1982; Singh and Sinclair, 1986).

The 50 samples were grouped arbitrarily according to the level of infection by Phomopsis spp.: high, with infection levels ranging from 33% to 77% (17 samples); medium, from 11% to 29% (17 samples); and low, from 0% to 9% infection (16 samples). Simple correlations were performed within each level of infection. The germination of seed (in standard test) was negatively correlated ($P \leq 0.001$) with percentage of infection by Phomopsis spp. (Table 8). Germination of lots with medium and low infection levels was not significantly affected by Phomopsis spp. Emergence in sand and TZG were not significantly affected by these fungi, as also illustrated by regression analyses (Figs. 3 and 4).

When the results of G, TZG, and ES were correlated against each other (Table 9) using seed lots with a high level of Phomopsis spp., the

Table 7. Ranges and means for several quality parameters for 50 samples of soybean seeds with emergence in sand > 70%.

Parameter	Range	Mean
	---------------%-------------	
Standard germination	36-96	75.7
Emergence in sand	71-99	82.3
Tz-germination	70-99	83.6
Tz-vigor	45-99	69.0
Phomopsis spp.	0-77	22.9
Phomopsis deep-seated	0-7	0.5
Damping-off	0-15	3.7

Table 8. Correlation coefficients for incidence of *Phomopsis* spp. in seed versus estimates of seed germination by three tests.

Parameter	Incidence of *Phomopsis* spp.[a] High	Medium	Low	Combined (n = 50)
Standard germination	-0.70[b]***	-0.36	-0.32	-0.70***
Emergence in sand	0.10	-0.35	-0.40	-0.04
Tz-germination	0.34	-0.33	-0.34	0.11

[a]Incidence: high = 33-77%, mean = 44.0, n = 17;
medium = 11-<33%, mean = 20.2%, n = 17;
low = 0-<11%, mean = 3.5%, n = 16.

[b]Correlation coefficient.

*** $P \leq 0.001$

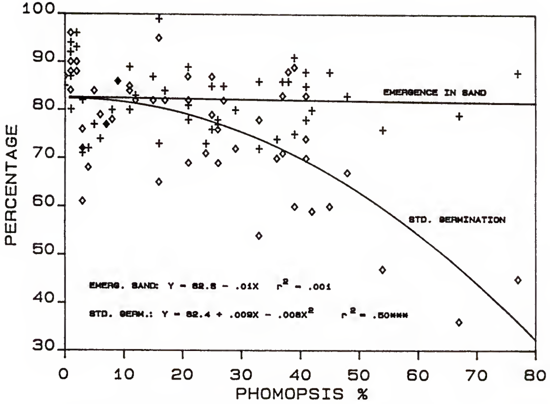


Fig. 3. Regression analyses for standard germination and emergence in sand versus incidence of <u>Phomopsis</u> spp. for 50 samples of soybean seed.

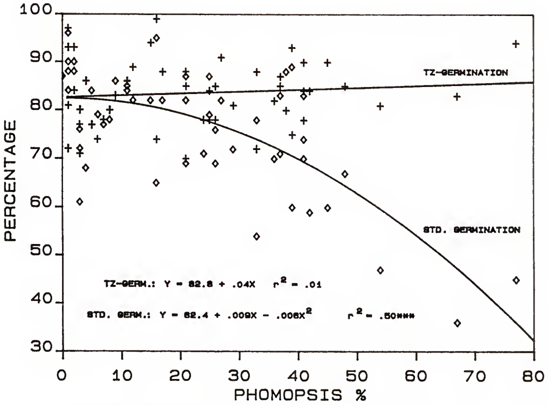


Fig. 4. Regression analyses for estimates of seed germination by tz and standard germination tests versus incidence of Phomopsis spp. for 50 samples of soybean seed.

Table 9. Coefficients of several correlations among estimates of germination by three tests, according to incidence of *Phomopsis* spp.

Parameters[b]	Incidence of *Phomopsis* spp.[a]			Combined (n = 50)
	High	Medium	Low	
G versus ES	0.46[c]	0.85***	0.73***	0.50***
G versus TZG	0.21	0.83***	0.52*	0.33*
ES versus TZG	0.82***	0.83***	0.78***	0.79***

[a]Incidence: high = 33-77%, mean = 44.0%, n = 17;
medium = 11-<33%, mean = 20.2%, n = 17;
low = 0-<11%, mean = 3.5%, n = 16.
[b]G: standard germination; ES: emergence in sand;
TZG: tz-germination.
[c]Correlation coefficient.
* $P \leq 0.05$; *** $P \leq 0.001$

correlations between G versus ES and G versus TZG were non-significant. However, significant correlation ($P \leq 0.001$) was observed for ES versus TZG, regardless of the level of infection.

The mean results for G, ES, and TZG, according to incidence of Phomopsis spp. are illustrated in Table 10. These tests are all measures of viability. Therefore, theoretically, the results obtained by these tests should be the same. This was not the case. If the seed samples with high incidence of Phomopsis spp. (mean of 44% infection) are considered, G produced the lowest viability value (66.7%), which was followed by ES (82.4%) and TZG (84.2%). As the incidence decreased, the difference between ES and G (and TZG and G) also decreased, being negligible for lots with low incidence of infected seed (Fig. 5). Similar results were reported in Brazil by Henning and Franca Neto (1980, 1985) and Franca Neto et al. (1985a), where Phomopsis spp. drastically reduced germination in the laboratory tests (rolled paper toweling), but did not affect emergence in soil or sand if the physiological quality of the seed was good, and if environmental conditions were adequate for fast emergence. In Brazil, and in Florida, as reported herein, seed lots with reduced germination (rolled towel) due to high levels of Phomopsis spp. could have high vigor and viability as determined by the tz and emergence-in-sand tests. One possible explanation for this is that infection occurring late in the season resulted in internal but not deep-seated seedcoat infection. In such cases, seed tested in rolled paper toweling have close contact between the infected seedcoats and cotyledons and embryonic axis, which increased the incidence of infected seedlings and dead seed.

Table 10. Means for estimates of seed germination by three tests for lots of soybean seeds with different incidence of *Phomopsis* spp.

Parameters	Incidence of *Phomopsis* spp.[a]			Mean (n = 50)
	High	Medium	Low	
	--------------------%	--------------------		
Standard germination	66.7	79.4	81.5	75.7
Emergence in sand	82.4	82.2	82.4	82.3
Tz-germination	84.2	84.4	82.1	83.6

[a]Incidence: high = 33-77%, mean = 44.0, n = 17;
medium = 11-<33%, mean = 20.2%, n = 17;
low = 0-<11%, mean = 3.5%, n = 16.

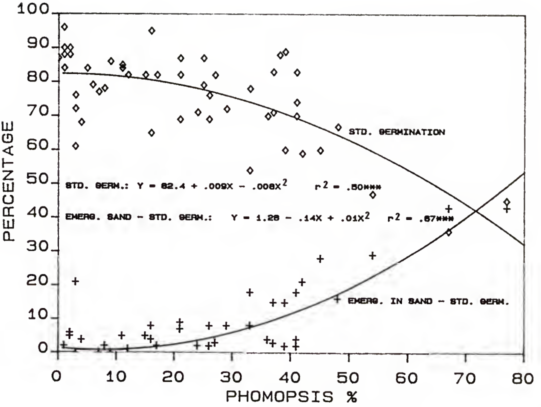


Fig. 5. Regression analyses for the difference between the results of emergence in sand and standard germination versus incidence of Phomopsis spp. and standard germination versus Phomopsis spp. for 50 samples of soybean seed.

Nonetheless, when seed were tested in sand or soil, the infected seedcoat was possibly left in or on the soil upon emergence, and in this way the seedling escaped much of the detrimental effects of infected seedcoats.

Loeffler et al. (1988) studied the influence of various factors on the use of bulk conductivity test as a rapid method for evaluating soybean seed viability. They reported that seed lots with high percentage of seed infection by Phomopsis spp. and Cercospora kikuchii and with intact seedcoats showed low conductivity and thus high potential seed quality. However, these seed had lower germination as determined by the rolled-paper-towel method, supporting perfectly the theory described in the previous paragraph about the response of highly infected seed by Phomopsis spp. on germination tests. Curiously, they overlooked the fact that tz-germination was higher than the standard germination for seed lots highly infected with Phomopsis spp., and concluded that "this lack of detection of infected seed poses an important limitation to the conductivity test". In contrast to their statements, high incidence of seed infection by these fungi may not pose an important limitation to the conductivity test, since it was demonstrated herein that, in situations like these, important limitations do exist, but with the standard germination test.

The minimum standard for germination for certified soybean seed in Florida is 80%. When rolled paper toweling was used to evaluate germination, 54% of the seed lots analyzed would have been discarded because the minimum level of 80% was not reached. Nevertheless, if germination were evaluated in sand, the level of discard would be

reduced to 36%. The difference between these values (18%) is the number of good quality seed lots that are being discarded, and this represents a great loss to the seed industry. Therefore, the standard germination test (rolled paper toweling) did not correctly evaluate the germination of soybean seed infected with <u>Phomopsis</u> spp. In Brazil, where more than 250 seed analysts were trained in soybean seed health and tz testing (Henning, et al., 1985), the blotter and tz tests and emergence in sand are routinely used in several seed laboratories (Franca Neto et al., 1985a). In the state of Parana, Brazil, in 1980, the use of these tests avoided the discard of 30,000 ton of good seed (17% of the seed needs for the state) that normally would be discarded if the rolled paper method alone were used (Henning and Franca Neto, 1985).

Additional information was obtained when analyses were performed on the set of data containing the 73 samples. Table 11 contains the ranges and means for several quality parameters for all samples. Again, deep-seated infection by <u>Phomopsis</u> spp. was low, and occurred only in 20% of the samples.

Correlation coefficients for <u>Phomopsis</u> spp. versus seedling damping-off (r = -0.20) and vigor by the tz test (r = 0.21) were non-significant. A highly significant negative correlation (r = -0.34) was observed for tz-vigor versus damping-off, supporting the conclusion that the lower the tz-vigor, the higher the level of seedling damping-off caused by certain fungi, such as <u>Phomopsis</u> spp. and <u>Colletotrichum truncatum</u>. In situations like this, the results obtained for TZG were slightly higher than for ES. The growth rate of these fungi is usually higher than the rate of germination and emergence of soybean seed with

Table 11. Ranges and means for several quality parameters for 73 samples of soybean seeds.

Parameter	Range	Mean
	---------------%--------------	
Standard germination	34-96	70.3
Emergence in sand	43-99	75.3
Tz-germination	55-99	78.9
Tz-vigor	30-99	62.5
Phomopsis spp.	0-77	20.1
Phomopsis deep-seated	0-10	0.7
Damping-off	0-15	4.6

medium or low vigor, resulting in higher indexes of infected seedlings and damping-off. Perhaps the lack of consistency in the results obtained by Kulik and Schoen (1981), regarding the effects of Phomopsis spp. on emergence of soybean seed, could be explained by probable differences in vigor of the seed lots studied.

Curiously, 27 years ago, Moore (1961) had already reported that TZG of soybean seed highly infected with "fungi" was higher than the germination as revealed by the standard method. Moore also stated that the question of whether the seed quality was better represented by the tz test or by the standard germination test would not be answered on that occasion. Hopefully, the answer for this question is provided herein.

Conclusions

The rolled-paper-towel test was not an accurate method to evaluate germination of soybean seed lots with high incidence of Phomopsis spp. The estimates of viability provided by this test were biased by the action of Phomopsis spp. from soybean seeds with high incidence of this fungus. The tz test, being a biochemical test, was not influenced by the presence of fungi within the soybean seedcoat. Emergence in sand was not influenced by seed infection by Phomopsis spp., if seed vigor, as expressed by tz test, was high. As the vigor determined by the tz test decreased, the rate of germination and emergence also decreased. Low-vigor seed samples were more vulnerable to the action of fungi in

the seedcoats, thus resulting in higher levels of seedling infection and damping-off.

The emergence-in-sand test provided estimates of germination for seed lots with high incidence of infection by Phomopsis spp. that more nearly simulated what would be expected in the field under ideal conditions. The results of germination from the tz test were similar to the results from the emergence-in-sand test. Therefore, the tz test also provided unbiased estimates of viability for seed lots with high incidence of seed infection by Phomopsis spp. Standardization of procedures for the emergence test in sand or in a soil substitute is needed to improve reliability and to decrease variation in results among laboratories, as reported by Franca Neto et al. (1986) and Skinner and Schroeder (1978). Finally, the use of the tz test is strongly recommended in studies involving physiological and pathological deterioration of soybean seed.

CHAPTER IV
EFFECTS OF *Colletotrichum truncatum* ON VIABILITY AND QUALITY OF SOYBEAN SEED

Colletotrichum truncatum (Schw.) Andrus & Moore is the causal organism of soybean anthracnose. This disease results in severe yield losses in warm and humid regions, such as the tropics and subtropics (Sinclair, 1982), where production of soybean is presently expanding. In India, anthracnose is considered the most serious soybean disease (Khare and Chacko, 1983). In the southern United States this disease caused estimated yield losses ranging from 0.1% to 7.0% in the growing seasons of 1982 to 1986 (Koldenhoven et al., 1983; Mulrooney, 1985, 1986, 1988). Estimates of maximal reductions in seed yield to anthracnose ranged from 16% to 26% in susceptible cultivars in Alabama (Backman et al., 1982). Yield losses of 30% to 50% were reported in Thailand and up to 100% in India (Sinclair, 1982). Although several soybean lines were rated as resistant to the pathogen, no germplasm accessions tested so far were totally free of disease (Manandhar et al. 1988).

C. truncatum is seedborne and seed-transmitted and may result in systemic infection of plants (Neergard, 1979). The pathogen overwinters in crop debris (Athow, 1973; Sinclair, 1982), and several weed species may also serve as sources of inoculum (Hartman et al., 1986; McLean and Roy, 1988).

In addition, the pathogen reduces seed quality. When infected seeds were planted, stands were reduced by preemergence and postemergence damping-off (McLean and Roy, 1988; Muchovej et al., 1980; Roy, 1982; Sinclair, 1982; Tiffany, 1951). Incidence of seed infection by C. truncatum is relatively low in temperate climates (McGee, 1986), but it may be high in the tropics, where levels above 50% were reported in Brazil by Franca Neto et al. (1984). Infection of soybean seed by C. truncatum was reported to be confined to the hourglass cells in the seedcoat (Kunwar et al., 1985; Sinclair, 1982), but it rarely infected embryo tissues (Rodriguez-Marcano and Sinclair, 1978; Schneider et al., 1974).

Germination of soybean seeds can be evaluated by different methods, such as emergence in sand, tetrazolium staining, and rolled paper toweling tests (Association of Official Seed Analysts - AOSA, 1981), the later being the simplest and most widely used method. The influence of certain fungi, such as Phomopsis spp. and Fusarium spp., on evaluating the viability of soybean seeds by these tests have been studied in detail by Henning and Franca Neto (1980, 1985), and as reported in Chapters II and III. Nevertheless, no report was found on possible effects of C. truncatum on evaluating the viability of soybean seeds by these methods. The use of a test that incorrectly evaluates germination of soybean seeds might result in great losses to the seed industry (Henning and Franca Neto, 1985).

The objectives of this study were to: a) determine the influence of C. truncatum on evaluating the viability of soybean seeds by the standard germination, emergence-in-sand, and tetrazolium tests; b)

determine the best method to evaluate germination of seeds infected with the pathogen; and c) investigate the depth of infection by this pathogen in soybean seed.

Materials and Methods

Seventy-three lots of soybean seed of 23 cultivars and five breeding lines produced in Florida in 1986 were evaluated for quality at the Agronomy Seed Laboratory of the University of Florida. Standard germination (rolled paper toweling), emergence-in-sand, tetrazolium (tz), and blotter tests were performed on the seed samples according to procedures described in Chapter III. Correlation and regression analyses were performed on the data set.

Results and Discussion

Ranges and means for several quality parameters for 73 seed samples are contained in Table 12. Seed lots varied widely in quality, ranging from as low as 34% to as high as 96% germination. Thirty percent of the seed samples (22 of the 73 seed samples) contained high levels (5% to 20%) of infection by *C. truncatum*. The pathogen was mainly localized in the seedcoat, supporting the results reported by Kunwar et al. (1985) and Sinclair (1982). Nevertheless, 50% of the 22 seed lots classified as having high levels of seed infection by the pathogen had 1% - 10% of the seeds with deep-seated (embryo) infection. This amount of embryo

Table 12. Ranges and means for several quality parameters for 73 samples of soybean seeds.

Parameter	Range	Mean
	-----------------%-----------------	
Standard germination	34-96	70.3
Emergence in sand	43-99	75.3
Tz-germination	55-99	78.9
Tz-vigor	30-99	62.5
C. truncatum	0-20	2.7
C. truncatum deep-seated	0-10	0.9
Damping-off	0-15	4.6

infection by *C. truncatum* was much more prevalent than that reported by Rodriguez-Marcano and Sinclair (1978) and Schneider et al. (1974).

The tests of standard germination (G), emergence in sand (ES), and tz-germination (TZG) measure the same parameter: seed viability. Therefore, theoretically, the results obtained by these tests should be the same. This was not the case, as illustrated by the mean values in Table 12. Seeds were shown to have highest percent germination when measured by tz test, less by ES, and least by G (LSD, $P \leq 0.05$).

Seed samples were arbitrarily grouped according to the level of seed infection by *C. truncatum*: high, with 5% to 20% incidence (22 samples), and low, from 0% to <5% incidence (51 samples). Simple correlations between several quality parameters were performed within the two levels of incidence. Highly significant ($P \leq 0.001$) positive correlations were obtained across all viability tests (G, ES, and TZG) for seed lots with low incidence of pathogen (Table 13). However, for samples with high incidence of seed infection with *C. truncatum*, highly significant correlation ($P \leq 0.001$) was obtained only for ES versus TZG. Thus, differences in viability were obtained by these tests for seeds with high incidence of this pathogen.

For the 73 seed samples, the significant ($P \leq 0.01$) negative correlations for *C. truncatum* versus G and versus ES (Table 14) established that the higher the incidence of seed infection by the pathogen, the lower the G and ES. However, germination as determined by the tz test was not significantly influenced by seed infection by this fungus.

Table 13. Correlations of three tests for soybean seed germination for two levels of seed infection by C. truncatum.

Seed test[a]	Incidence of C. truncatum[b]	
	High	Low
G versus ES	0.40[c]	0.71***
G versus TZG	0.15	0.64***
ES versus TZG	0.72***	0.90***

[a]G: standard germination; ES: emergence in sand; TZG: tz-germination.
[b]Incidence: high = 5-20%, mean = 7.7%, n = 22;
low = 0-<5%, mean = 0.7%, n = 51.
[c]Correlation coefficient.
*** $P \leq 0.001$

Table 14. Correlation coefficients for soybean seed infection by _C. truncatum_ versus several quality parameters for 73 seed samples.

Parameter	Correlation coefficients
Standard germination	-0.30**
Emergence in sand	-0.33**
Tz-germination	-0.08
C. truncatum deep-seated	0.59***
Damping-off	0.64***

** $P \leq 0.01$; *** $P \leq 0.001$

Correlations for seed infection by *C. truncatum* versus deep-seated (embryo) infection, and damping-off were highly significant ($P \leq 0.001$). Indications are that the pathogen was a major source of seedling damping-off, as previously reported by Mclean and Roy (1988), Muchovej et al. (1980), Roy (1982), Sinclair (1982) and Tiffany (1951).

Means for several quality parameters, for each level of seed infection by *C. truncatum*, are illustrated in Table 15. Again, it is noted that the amount of damping-off was minimal for low levels of seed infection by the pathogen. The mean incidence of seed infection by *Phomopsis* spp. was approximately the same (20 - 21%) for all samples. The results of viability as determined by G, ES, and TZG for lots with high levels of *C. truncatum* and for all samples were not the same (LSD, $P \leq 0.05$). According to several reports (Franca Neto et al., 1985a; Henning and Franca Neto, 1980, 1985), and as reported in Chapters II and III, *Phomopsis* spp. can drastically reduce germination in the laboratory (rolled paper toweling), but may not affect emergence in the sand test or in the field, if the physiological quality of the seed is good, and conditions are favorable for fast emergence. In Brazil, as explained by Henning and Franca Neto (1980, 1985) and in Florida, as illustrated in Chapters II and III, seed lots with reduced germination (rolled paper) due to high levels of *Phomopsis* spp. could have high vigor and viability as determined by emergence in sand and tz test. Infection by *Phomopsis* spp. that occurs late in the season frequently results in internal but not deep-seated seedcoat infection. In such cases, when seeds are tested in rolled paper toweling there is close contact between the infected seedcoats and cotyledons, which increases

Table 15. Means for several seed quality characteristics for lots of soybean seeds infected by _C_. _truncatum_.

Parameter	Incidence of _C_. _truncatum_[a]	
	High	Low
	--------------%--------------	
_C_. _truncatum_ deep-seated	2.1	0.3
Phomopsis spp.	21.8	20.4
Standard germination	67.1	72.9
Emergence in sand	74.1	77.5
Tz-germination	80.6	79.5
Damping-off	6.4	3.7

[a]Incidence: high = 5-20%, mean = 7.7%, n = 22;
low = 0-<5%, mean = 0.7%, n = 51;

the level of infected seedlings and dead seeds. In contrast, when seeds are tested in sand or soil, seedlings leave the infected seedcoat in or on the soil upon emergence, and in this way the seedlings escape much of the detrimental effects of infected seedcoats. Like Phomopsis spp., C. truncatum also reduces germination, but only slightly. The standard germination obtained for samples with high incidence by C. truncatum was 67.1% compared to 72.9% for samples with low incidence (Table 15).

As reported previously, the results obtained by ES should be similar to those from TZG. However, TZG was statistically higher (LSD, $P \leq 0.05$), especially for lots with high incidence by C. truncatum. Since the tz test is a biochemical test it was probably not affected by the presence of fungi within the seed, thus resulting in the highest values. Emergence in sand was apparently not influenced by superficial infections by Phomopsis spp., but could have been affected by C. truncatum, which may have caused seedling damping-off, as illustrated in Table 15. Results of ES and TZG for samples with low C. truncatum statistically did not differ (LSD, $P \leq$).05). Again, standard germination, which had the lowest values, may have been affected by the presence of both fungi.

The seed lots were also arbitrarily grouped according to the tz-vigor level: high, with vigor levels above 70% (23 samples); medium, from 50% to <70% (33 samples); and low, from 30% to <50% (17 samples). Correlations between quality parameters within each tz-vigor level are listed in Table 16. The non-significant correlation coefficients for C. truncatum versus tz-vigor support the conclusion that the tz test was not affected by seed infection by the pathogen. The presence of C.

Table 16. Correlation of incidence of soybean seeds with *C. truncatum* versus tz-vigor and versus seedling damping-off among seed lots with different vigor.

Parameters[a]	Tz-vigor level[b]			Combined
	High	Medium	Low	(n = 73)
Ct versus TZG	-0.19[c]	-0.01	0.27	-0.08
Ct versus DO	0.77***	0.61***	0.59*	0.64**

[a]Ct: seed infection with *C. truncatum*; TZV: tz-vigor;
DO: damping-off.
[b]Vigor level: high = 70-99%, mean = 80.0%, n = 23;
medium = 50-<70%, mean = 61.0%, n = 33;
low = 30-<50%, mean = 42.0%, n = 17.
[c]Correlation coefficient.
** $P \leq 0.01$; *** $P \leq 0.001$

truncatum on seed was significantly correlated ($P \leq 0.01 - 0.001$) with damping-off for all tz-vigor levels. Thus, seed infection by this pathogen is a significant source of damping-off, regardless of seed vigor. *Phomopsis* spp., as reported in Chapter III, caused seedling damping-off only for low vigor seed lots. The trends of data in Table 16 are illustrated by the means of several quality parameters included in Table 17.

Conclusions

Soybean seed infection by *C. truncatum* was mainly confined to the seedcoat. However, embryo infection was not rare. Additionally, seed infection by this pathogen was a major source of seedling damping-off, regardless of seed vigor.

The detection of seed viability by the tz test was not influenced by the amount of seed infected by *C. truncatum*. Due to the possibility of occurrence of damping-off, if seeds are planted, the tz test would overestimate the viability of soybean seed lots infected with high incidence of this pathogen. In contrast, the rolled paper toweling method underestimated viability for seed lots with high incidence of this fungus. Emergence in sand provided more reliable estimates of viability for seed lots infected with *C. truncatum*.

The conclusions in this paper were based on analyses performed on seed samples that were not treated with any fungicide. Additionally, this experiment was not designed to study the dissemination of the

Table 17. Means for several seed quality parameters for lots of soybean seeds with different vigor.

Parameter	Tz-vigor level[a]		
	High	Medium	Low
	-------------------%-------------------		
C. truncatum	2.9	2.4	3.9
C. truncatum deep-seated	0.6	1.0	0.8
Standard germination	79.4	70.9	54.9
Emergence in sand	85.3	77.2	55.9
Tz-germination	89.8	78.1	64.9
Damping-off	3.6	4.3	6.4

[a]Tz-vigor level: high = 70-99%, mean = 80.0%, n = 23;
medium = 50-<70%, mean = 61.0%, n = 33;
low = 30-<50%, mean = 42.0%, n = 17.

pathogen or the control of the disease. Further investigations are necessary to determine the effects of fungicide treatment of soybean seed infected with C. truncatum on seed quality, and on control and spread of the pathogen.

CHAPTER V
EFFECTS OF *Cercospora kikuchii* ON THE QUALITY OF SOYBEAN SEED

Cercospora kikuchii (Matsu. & Tomoyasu) Gardner is the causal organism of purple seed stain in soybeans (Lehman, 1950). Infected seed show typical purple discolorations, but symptomless seed may also carry the pathogen (Agarwal, 1981; Sinclair, 1982). Soybean pods, stems, and leaves may also be infected by the fungus, resulting in Cercospora leaf blight (Walters, 1980). The fungus overwinters in crop residues, which is the major source of inoculum. The occurrence of purple seed stain is higher when soybean seeds mature under warm and humid environments (Sinclair, 1982), such as in the tropics.

Seedborne inoculum had little effect on disease severity and harvest losses on areas previously cultivated with soybeans (Franca Neto et al., 1983; McGee et al., 1980; Wilcox and Abney, 1973). An antagonistic effect between *C. kikuchii* and other seedborne pathogens was reported by Roy and Abney (1977) and Hepperly and Sinclair (1981): soybean seed infected with *C. kikuchii* had lower incidence of *Phomopsis* spp. and *Fusarium* spp.

In histopathological studies, Ilyas et al. (1975), and Singh and Sinclair (1986) showed that hyphae of *C. kikuchii* were generally

localized in the seedcoat. Occasionally hyphae were found in the cotyledons and rarely in the embryonic axis.

Purple seed staining has not been reported to cause any yield losses to soybeans (Franca Neto et al., 1983; Sinclair, 1982; Wilcox and Abney, 1973). However, the role of C. kikuchii in soybean seed quality seems to be poorly understood, since the reports on its effects on soybean seed quality have provided inconsistent conclusions. Lehman (1950) observed that the infection by C. kikuchii did not result in reduced germination, but caused some postemergence damping-off. Murakishi (1951) reported a reduction of up to 25% in germination due to seed infection by C. kikuchii. Wilcox and Abney (1973) showed that soybean seeds infected with the pathogen had lower germination in sand (up to 5%), and emergence in the field (up to 18%). Similar reduction in emergence in soil was reported by Agarwal (1981). Nevertheless, Franca Neto et al. (1983), McGee et al. (1980), Sherwin and Kreitlow (1952), and TeKrony et al. (1985) presented no significant effect of infection by this fungus on germination and emergence of soybean seeds. Loeffler et al. (1988) reported no effect of C. kikuchii on the quality of soybean seeds assessed by the bulk conductivity test. Inconsistent conclusions were also obtained by Hepperly and Sinclair (1981), who performed pathological studies on soybean seeds produced in Puerto Rico. No detrimental effects of seed infection by C. kikuchii were observed on germination when soybean seeds were harvested at harvest maturity. However, negative effects on germination were observed after a 30-day delay in harvest. Further studies on this subject were suggested by these researchers.

The objectives of the present research were: a) to study the effects of C. kikuchii on the quality of soybean seeds produced in Florida; b) to explain why various researchers reached different conclusions about the effects of C. kikuchii on the quality of soybean seed; and c) to draw a valid conclusion consistent with all data published so far about the subject.

Materials and Methods

Seventy-three soybean seed samples of 23 cultivars and five breeding lines produced in Florida in 1986 were evaluated for quality at the Agronomy Seed Laboratory of the University of Florida. Standard germination, emergence-in-sand, tetrazolium (tz), and blotter tests were performed on the seed samples according to procedures described in Chapter III.

Correlation analyses were performed on four sets of data: all 73 seed samples; 14 samples with high incidence (7% to 33%) by C. kikuchii; 16 samples with medium incidence (4% to 6%); and 43 samples with low incidence (0% to 3%) by the pathogen.

Results and Discussion

Ranges and means for several quality parameters for all 73 seed samples are given in Table 18. Seed samples ranged from low quality (34% germination, 30% tz-vigor) to very high quality (96% germination, 99% tz-vigor). Despite the warm and humid climatic conditions in

Table 18. Ranges and means for several quality parameters for 73 samples of soybean seeds.

Parameter	Range	Mean
	--------------%--------------	
Standard germination	34-96	70.30
Emergence in sand	43-99	75.30
Tz-germination	55-99	78.90
Tz-vigor	30-99	62.50
<u>C</u>. <u>kikuchii</u>	0-33	4.80
<u>C</u>. <u>kikuchii</u> deep-seated	0-2	0.07
Damping-off	0-15	4.60
<u>Phomopsis</u> spp.	0-77	20.10

Florida during the period of seed maturation, the incidence of seed with C. kikuchii for the 73 seed samples was low, with an average of 4.8%. Only seventeen percent of the seed samples (14 samples) contained high incidence (7% to 33%) of C. kikuchii. The pathogen was almost exclusively restricted to the seedcoat, since only four samples had deep-seated (embryo) infection, with a maximum incidence of embryo infection of only 2%. Ilyas et al. (1975) and Singh and Sinclair (1986) also found that hyphae of C. kikuchii were generally localized in the seedcoat.

Means for several quality parameters, according to the level of infection by C. kikuchii, are included on Table 19. The results of viability, as determined by the standard germination test (rolled paper toweling), emergence in sand, and tz-germination, theoretically should be the same, but this was not the case: standard germination statistically (LSD, $P \leq 0.05$) exhibited the lowest values, followed by emergence in sand and then tz-germination. These differences were due to the effects of seed infection by Phomopsis spp. and Colletotrichum truncatum (Schw) Andrus & Moore. These fungi may affect the reliability of some of these tests, especially the standard germination test. These effects are explained in Chapters III and IV.

Seed samples with high incidence of C. kikuchii had the highest germination, emergence in sand, and tz-germination, and the lowest incidence of Phomopsis spp., when compared to seed samples with medium and low incidence of seed with the pathogen (Table 19). A significant negative correlation (-0.35) was obtained between incidence of seed infection with Phomopsis spp. versus C. kikuchii for seed samples

Table 19. Means for several seed quality characteristics for lots of soybean seeds infected by C. kikuchii.

Parameter	Incidence of C. kikuchii[a]		
	High	Medium	Low
	------------------- % -------------------		
C. kikuchii deep-seated	0.14	0.06	0.05
Standard germination	76.80	70.00	67.80
Emergence in sand	79.30	74.80	73.70
Tz-germination	83.60	78.90	77.30
Damping-off	4.20	5.60	4.20
Phomopsis spp.	13.30	23.40	21.70

[a]Incidence: high = 7-33%, mean = 14.7%, n = 14;
medium = 4-<7%, mean = 5.1%, n = 16;
low = 0-<4%, mean = 1.4%, n = 43.

with high incidence of C. kikuchii (Table 20). Antagonism between these two fungi has been reported by Hepperly and Sinclair (1981), and Roy and Abney (1977). For this reason Roy and Abney (1977) suggested the use of C. kikuchii as an agent for the biological control of Phomopsis seed infection. This suggestion may not be feasible, since C. kikuchii, as reported by Walters (1980), can also cause Cercospora leaf blight, which can result in yield losses to soybeans.

No significant ($P \leq 0.05$) effects of seed infection by C. kikuchii on standard germination, emergence in sand, tz-germination, tz-vigor and damping-off were detected by the correlation analyses (Table 20), for all 73 seed samples, or within any of the three groupings, according to the level of incidence of the pathogen. These results provide additional support to the conclusions reported by Franca Neto et al. (1983), McGee et al. (1980), Sherwin and Kreitlow (1952), and TeKrony et al. (1985), that seed infection by this fungus does not greatly affect germination or emergence of soybean seeds.

The limited results obtained in the present study, together with the ones reported by Franca Neto et al. (1983), McGee et al. (1980), Sherwin and Kreitlow (1952), and TeKrony et al. (1985), which presented no significant negative effects of seed infection of C. kikuchii on germination and emergence of soybean seeds, do not explain the reasons for the inconsistent conclusions published so far about the effects of C. kikuchii on the quality of soybean seed. However, the following discussion may provide the reader with new insights that, hopefully, will clarify this matter. All the reports that indicated a deleterious

Table 20. Correlation of seed germination, seedling damping-off, seed vigor, and seed infection by *Phomopsis* spp. versus incidence of *C. kikuchii* on soybean seeds.

Parameter	Incidence of *C. kikuchii*[a]			Combined
	High	Medium	Low	(n = 73)
Standard germination	0.12[b]	0.06	0.15	0.22
Emergence in sand	0.26	0.27	0.21	0.20
Tz-germination	-0.05	0.45	0.23	0.21
Tz-vigor	-0.01	0.33	0.16	0.25
Damping-off	0.05	0.36	-0.10	0.02
Phomopsis spp.	-0.35*	0.32	-0.01	-0.14

[a]Incidence: high = 7-33%, mean = 14.7%, n = 14;
medium = 4-<7%, mean = 5.1%, n = 16;
low = 0-<4%, mean = 1.4%, n = 43.

[b]Correlation coefficient.

* $P \leq 0.05$

effect of seed infection by C. kikuchii on the quality of soybean seed (Agarwal, 1981; Hepperly and Sinclair, 1981; Lehman, 1950; Murakishi, 1951; and Wilcox and Abney, 1973) had a common methodological approach which might have provided maximum opportunity for a detrimental effect. In these studies soybean seeds were artificially separated into two classes: those with symptoms (100% purple stained seed), and those without symptoms of purple seed stain. Natural populations of seed samples with 100% purple stained seeds have not been reported in the literature. The highest natural infection level was 57% as reported by McGee et al. (1980), and even then no detrimental effects were shown. In addition to the use of this artificial situation, Hepperly and Sinclair (1981), and Murakishi (1951) evaluated seed germination on potato-dextrose agar (PDA), thus providing ideal conditions for the development of seedborne fungi. The Rules for Testing Seeds (Association of Official Seed Analysts, 1981) do not prescribe PDA as an appropriate substrate for the standard germination test. Furthermore, under these conditions, maximum opportunity for a detrimental effect was provided again, and the effect of C. kikuchii on the germination of soybean seeds might have been overestimated. With the exception of Sherwin and Kreitlow (1952), all the workers who reported no significant effect of seed infection by C. kikuchii on soybean seed quality, including the study reported herein, used normal field incidence of C. kikuchii.

Conclusions

Soybean seed infection by *C. kikuchii* was almost exclusively confined to the seedcoat. An antagonistic effect was observed between *C. kikuchii* and *Phomopsis* spp. infecting soybean seeds: the higher the level of seed infection by *C. kikuchii*, the lower the seed infection by *Phomopsis* spp. No detrimental effects of seed infection of this pathogen were observed on germination, emergence, and vigor of soybean seeds with the natural field incidence of purple-stained seeds used in this study.

Inconsistent conclusions obtained by various researchers apparently were associated with different methodological approaches. Detrimental effects of seed infection by *C. kikuchii* on the quality of soybean seeds were reported by those researchers who provided maximum opportunity for a detrimental effect, i. e., 100% purple stained seeds were compared to symptomless seeds. Whereas, researchers who used normal field infection levels of *C. kikuchii* found no significant effect.

CHAPTER VI
THE TETRAZOLIUM TEST: A USEFUL TOOL ON PRODUCTION AND QUALITY RESEARCH OF SOYBEAN SEED

The production and utilization of high quality seeds of soybean is one of the most important and basic keys for the successful production of the crop. To achieve these requisites, the quality control program of the soybean seed industry must be versatile and dynamic, thus promptly providing accurate results. Several determinations, such as varietal and physical purity, moisture content, and level of mechanical damage can be evaluated within minutes, thereby partially fulfilling these requirements.

The time-consuming determination of viability by the standard germination test presents a serious limitation to the entire process of decision making within the seed industry. In addition to this limitation, the standard germination test provides very restricted information that is most reliable when ideal conditions are provided to the seed. For example, the results provided by the test can be severely influenced by seed infection by different pathogens, such as *Phomopsis* spp., *Fusarium semitectum*, and *Colletotrichum truncatum* (Henning and Franca Neto, 1980, 1985; Kulik and Schoen, 1981; TeKrony et al., 1984; Thomison et al., 1988), and as illustrated in Chapters III and IV.

Faster and more comprehensive results than those provided by the standard germination test are provided by the tetrazolium (tz) test. In addition to germination potential, the test also provides a vigor index, and reveals causes of seed weakness, such as mechanical damage, field and storage deterioration, stink bug damage, and damage to heat and frost.

Several aspects about the tz testing of soybean seed will be considered in this paper: a) the major events and accomplishments which contributed to the development and perfecting of the test; b) the basic principles of the test; c) needed equipment and supplies; d) procedures for seed preparation and evaluation; e) basis for the correct interpretation of the results; f) advantages and limitations of the test; and g) accuracy of the results. In addition, some research data obtained from soybean seed lots produced in Florida will be presented.

History

"The successful development of the tz test represents the accomplishment of many milestones in the history of seed research and in the attainment of new insights into seed life" (Moore, 1985, p. 2). More detailed reviews about the history of the tz test were published by Cottrell (1948), Delouche et al. (1962), Gadd (1950), Isely (1952), Lakon (1953), Lindenbein (1965), and Moore (1962a, 1966, 1969, 1976). A brief summary of its history and major accomplishments follow.

Many early testing methods used certain seed characteristics, such as color, appearance, volumetric weight, rate of imbibition, electrical

conductivity, density, and heat of respiration, to estimate seed viability. Nevertheless, the results obtained by these methods were not accurate. During the early 1920s the activity of certain enzymes, such as peroxidase, catalase, oxidase, reductase, and phenolase received special attention, but the lack of success of enzyme detection tests was likely due to the fact that individual seeds were not evaluated. Several stains, such as indigo carmine, sulfuric acid, methylene blue, neutral red, and malachite green were also used. Here too, the lack of precision of the tests was the major problem.

As reported by Moore (1969), the first successful attempts to evaluate seed viability by vital stains were accomplished by Turina of Yugoslavia in 1922, and by Neljubow of Russia in 1925. Turina worked with the reduction of tellurium and selenium salts in seed cells, and Neljubow reported some success with indigo carmine. Hasegawa of Japan, working with tree seed in the early 1930s, improved the application of selenium and tellurium salts in the staining of seed embryos. Most of his work was published in Japanese, thus making his achievements unaccessible to most of the scientific community. Part of his studies was widely publicized after he released some of his findings in English (Hasegawa, 1935), and in German, during a meeting of the International Seed Testing Association in Europe. During this trip, Hasegawa revealed certain details of his testing procedures to the German scientist, F.E. Eidmann, who also improved the selenium method (Moore, 1969).

Lakon, from Hohenhein, Germany, who had shown a great interest in seed physiology since the early 1920s, perfected the selenium method developed by Hasegawa and Eidmann, culminating with the development of

the topographical selenium method (Lakon, 1940). After he realized the poisonous characteristics of selenium for laboratory use, Lakon searched for a similar but non-toxic salt that could be used for the same purpose. After Kuhn and Jerchel (1941), as pointed out by Cottrell (1948) and Isely (1952), first called attention to the reduction of tetrazolium compounds in living tissues, Lakon tested several of these salts and concluded that the 2,3,5, triphenyl tetrazolium chloride was the most appropriate for the topographical test. Lakon developed his test on several cereal crops and corn.

As reported by Moore (1976), knowledge of the existence and merits of the tz testing was first received in America in 1945 from U.S. Military personnel, who investigated research activities in Germany after World War II. The first research work with the tz test conducted in the U.S. was published by Porter, Durrell, and Romm (1947), from Iowa State University. Other pioneer studies in America, as pointed out by Moore (1976), were published in 1948 by Flemion and Poole, from the Boyce Thompson Institute of Yonkers, New York, by Goodsell, from Pioneer Hi-Bred Corn Company of Johnston, Iowa, and by Bennett, from Iowa State University. Substantial achievements and improvements on the tz test were achieved during the 1950s. Several researchers from different U.S. universities made outstanding contributions. They include Isely, Bass, Smith, and Throneberry from Iowa State University, and Parker from the University of Idaho. In the 1960s, important developments concerning the practical application of the tz test were obtained by Delouche, Still, Raspet, and Leinhard from Mississippi State University, who published the first tz test handbook for a large number of seed species

(Delouche et al., 1962). Jensen, Pierpoint, Hayes, and Grabe from Oregon State Seed Laboratory and Copeland, Bruce, and Midyette from Virginia, also provided important improvements on the test.

In 1970 another milestone was achieved. The use of the tz test was accepted by the Association of Official Seed Analysts (AOSA), with the release of the "Tetrazolium Testing Handbook" (Grabe, 1970). In 1983 the AOSA published the "Seed Vigor Testing Handbook" (AOSA, 1983), which compiled important information about the methodology of the tz test for soybean, cotton (Gossypium hirsutum L.), corn (Zea mays L.), and wheat (Triticum aestivum L.).

Special recognition and tribute are due to R.P. Moore from the North Carolina State Seed Laboratory. Between 1955 and 1985 he released more than 80 publications about the tz test, and edited the outstanding "Handbook on Tetrazolium Testing" (Moore, 1985), published by the International Seed Testing Association. This publication contains details and procedures for the application of the test to more than 650 species.

The tz test was also successfully accepted and used in several other countries. The test was introduced in Brazil by several seed technologists that were trained at Mississippi State University. The test was perfected for soybean seed, a very important crop to Brazil, the second largest producer of soybean in the world. L.A.G. Pereira refined the methodology of the test by providing a detailed description of the different classes for seed classification. Today, the tz test is routinely and successfully employed in several seed laboratories in Brazil (Franca Neto et al., 1985a). This achievement came after

intensive training of more than 250 seed analysts at the National Center for Soybean Research, as reported by Henning et al. (1985), and after the release of a detailed handbook about the test specifically directed towards soybean (Franca Neto et al., 1985b).

Principles of the Test

The tz test relies on the activity of the dehydrogenase enzymes (AOSA, 1983; Bulat, 1961; Copeland et al., 1959; Moore, 1973; Smith, 1952; Smith and Throneberry, 1951), which catalyze the reactions in glycolysis and the citric acid cycle. These enzymes, particularly the malic acid dehydrogenase, carry out the reduction of the tz-salt in living tissues. When a soybean seed is immersed in the colorless tz-solution, the tz penetrates into the seed tissues, where the tz interferes with the reduction processes of the living cells by accepting a hydrogen ion. In the reduced form, the tz-salt is a red-colored, stable, non-diffusible substance, called formazan. If the tissue is vigorous, a normal faint red color will result; if it is weak, an intensive red will develop; if it is dead, no reduction will occur, and the dead tissue will contrast as white (non-colored) with the stained living tissue. These color differences, together with the knowledge of several seed features, permit an assessment of the presence, location, and nature of disturbances within embryo tissues (Moore, 1973).

Equipments and Supplies

Among several tz-salts, the use of 2,3,5 triphenyl tetrazolium chloride is suggested. A staining oven or germinator with temperature capability of 40°C is required. Other supplies consist of Petri dishes, 50-mL beakers or plastic cups, single edge razor blades, and a refrigerator, for storage of the tz-solutions and stained samples. A magnifying lens (6X to 10X) with fluorescent lamps are required for the examination of the soybean seed samples. Germination paper is necessary for conditioning the seed. Dark plastic or glass bottles are needed to store the tz-solutions. The use of metallic flasks must be avoided, since the tz-salt might be reduced when in contact with certain metals (Bulat, 1961).

Procedure

The use of a 0.075% solution of the tz-salt is recommended, which is in contrast with most literature published so far, in which the authors suggest the use of 0.5% to 1.0% solutions (AOSA, 1983; Delouche et al., 1962; Grabe, 1970; Moore, 1985). The use of a more dilute solution permits the seed analyst to visualize some minor damages that normally would not be detected if a less dilute solution were used. In addition, the use of 0.075% solution is cost saving: 190 seed samples of 100 seeds each can be analyzed with only 10 g of the salt, as compared to merely 30 samples if a 0.5% solution were used.

The use of 100 seeds (two replicates of 50 seeds each) per sample is necessary for tz test (AOSA, 1983; Franca Neto et al., 1985b; Moore, 1973). No special preparation of the seeds is required for conditioning. Seeds are kept overnight in a moist germination paper towel at 25°C for 16 h. This operation permits the seed to imbibe water slowly, thus activating the germination process.

After conditioning, the seeds are placed in plastic cups or beakers, and covered with the 0.075% tz-solution. These cups are then placed in an oven or germinator at 40°C for 2.5 to 3.0 h. After staining, the seeds are rinsed with tap water several times to stop the staining reaction. It is important to keep the seeds submerged in water to avoid dehydration. Stained seeds can be kept in a refrigerator for the period of up to 12 h before they are evaluated.

The seedcoats of dark coated varieties of soybean are impermeable to the tz-solution. Therefore, seedcoats must be removed from these seeds after conditioning, and before staining begins.

Special care must be exercised to store the tz-solutions, and to stain the seeds in darkness, since the reagent is light-sensitive (Lakon, 1949).

Seed Evaluation

Unlike the requirement of very simple and inexpensive equipment and supplies, the tz test demands the expertise of a well-trained seed analyst. Knowledge of seed and seedling structures is a major element required of the analyst. Experience, judgement, and perhaps imagination

are also necessary for the analyst to visualize the kinds of seedling abnormalities revealed by the tz test. Accuracy and reliability are dependent upon the knowledge of all test techniques.

As pointed out by Moore (1985), there are three basic objectives of the seed evaluation: a) to determine the potential of germination of a seed lot under the most ideal conditions; b) to stratify the seed into different categories of viability in order to report a vigor test rating; and c) to diagnose the possible causes of seed weaknesses that resulted in the loss of viability. The first two objectives can be achieved by the interpretation of four basic characteristics: condition and color of the tissue after staining, and position and extent of damage. The ability of the seed analyst to recognize the symptoms of different kinds of damage is imperative for the correct diagnosis of the causes of lost viability.

Individual seeds are examined through a magnifying lens (6X to 10X) under fluorescent light. A single edge razor blade is used to cut the seed through the seedcoat and longitudinally through the midsection of the embryonic axis (Fig. 6). Care must be exercised to section the embryonic axis exactly at its midsection. If the cut is off-center, the evaluation of the condition of the embryonic axis should be made on the seed half which contains the larger portion of the axis, exposing its midsection after additional slicing. After the seed is sectioned, the seed halves are separated and the seedcoat is removed to expose the outer surface of the cotyledons. The analyst should observe the inner and outer surfaces of the cotyledons, inspecting for all types of defects. Special care must be applied during the evaluation of the

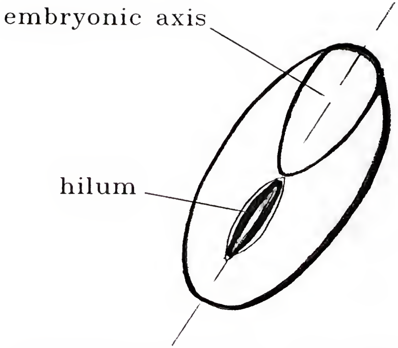


Fig. 6. Illustration of cutting site, through the midsection of a soybean seed.

radicle-hypocotyl axis. This axis is composed of two kinds of tissues: the cortex, and the stele or vascular cylinder (Fig. 7). The stele is the most critical structure of the radicle-hypocotyl axis. As a general rule, if damage occurs on this axis, but it is not deep enough to injure the stele, the seed may be considered viable. However, if the damage harms the stele, then the seed is rated as non-viable (Fig. 8).

There are three basic color intensities: faint red carmine, dark red carmine, and white. Faint red carmine identifies a vigorous and healthy tissue. According to Moore (1985), sound tissues tend to stain gradually and uniformly from the exposed surface inward. A high level of turgidity is present in moist tissues. Dark red carmine is typical of weak tissues, which are in the process of deterioration. Upon exposure to dry air, these tissues will lose turgidity more rapidly than vigorous tissues (Moore, 1985). White identifies dead tissue, which lacks the enzymatic activity necessary for the production of formazan. Dead tissues are usually flaccid, and commonly chalky-white, but may show yellowish, greenish or grayish tones, especially when the tissue has suffered stink bug damage. On some rare occasions, some dead tissues may show a mottled red intensity, due to high microbial activity. However, these tissues are easily differentiated from weak tissues due to their extreme flaccidity and friability. It should be emphasized that after the seed is sectioned, the internal surfaces of live cotyledons are normally white, due to the lack of diffusion of the tz-solution to the inner tissues of the cotyledons. Moore (1985) reported that viable non-stained tissues are usually turgid, lustrous, and pinkish- or yellowish-white.

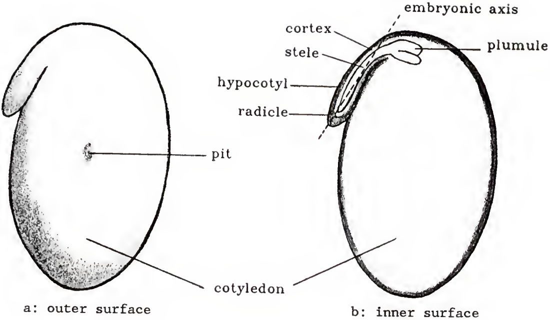


Fig. 7. Structures of an imbibed soybean seed with the seedcoat removed: a) outer surface; b) inner surface, after longitudinal sectioning.

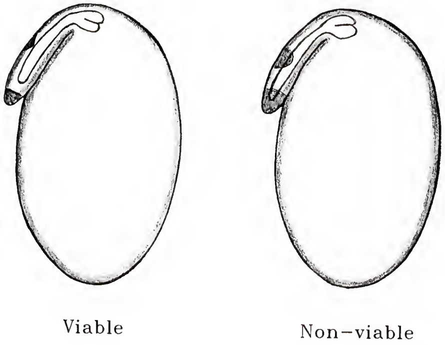


Fig. 8. Illustration of different severities of damage to the radicle-hypocotyl axis.

The position and extent of a certain damage are characteristics of crucial importance for the correct evaluation of the seed, and must be considered in combination. For example, in soybeans, a small area of damage caused by the feeding of a stink bug on the hypocotyl, which damages the stele, will result in more serious consequences than a large area of damage by stink bug feeding on the lower half of a cotyledon, far away from the embryonic axis.

Diagnosis of the Causes of Soybean Seed Deterioration

Several factors contribute to lower the quality of soybean seed. The major ones, as described by Franca Neto (1984), and Moore (1960, 1962a, 1973) are mechanical and weathering damages, stink bug damage, heat and drought damage, and freeze injury. Each kind of injury is associated with very typical staining patterns and tissue characteristics, that are briefly illustrated as below.

Mechanical damages (Fig. 9) result from physical impacts especially during harvesting, threshing, processing, drying, and sowing of soybean seed. There are three types of mechanical injury which are easily identified by the tz test: cracks, splits, and bruises. The latter is typically identified by the presence of dark-red speckles, if of recent occurrence, or by white and flaccid tissues, if not recent. It is very common for an inexperienced analyst to mistake the pit (Fig. 7) for a mechanical injury. The pit is composed of a group of specialized cells on the abaxial surface of the cotyledons in direct apposition to the

Fig. 9. Soybean seeds with typical symptoms of mechanical damage. Left: cracked seed; right: bruised seed.

seedcoat antipit, an enlarged layer of cells on the ventral surface of the seedcoat (Yaklich et al., 1984, 1986).

Weathering damage, as described by Franca Neto (1984), Moore (1973), and Pereira and Andrews (1985) is the result of the exposure of soybean seeds to alternate wetting-drying cycles before harvest. Damages will be of greater magnitude if these conditions occur in warm environments. Weathered seeds often show characteristic wrinkles on the cotyledonary region opposite the hilum, or on the hypocotyl axis. If stained, seeds will reveal the presence of dark-red and white patches on embryonic tissues just beneath these wrinkles. Frequently these lesions are associated with infection by certain fungi. The lesions can be deep, and if the stele is damaged, or if more than 50% of the cotyledonary tissue is destroyed, the seed is considered non-viable. A very typical characteristic of most weathered seeds is the symmetry of the lesions on both seed halves (Fig. 10).

Stink bug damage can seriously affect the soybean seed quality. Several species of stink bugs attack soybean in the U.S.A. The green stink bug, Acrosternum hilare (Say) and the southern green stink bug, Nezara viridula (L.), are the most important (Turnipseed and Kogan, 1976). When stink bugs feed on soybean seeds they also inoculate them with the yeast fungus Nematospora coryli Peglion (Sinclair, 1982). Colonization of this fungus in seed tissue often causes severe losses of seed viability and vigor (Bowling, 1980; Villas Boas et al., 1982). This infection results in characteristic circular, sometimes shrunken, and deep lesions (Fig. 11). Lesioned tissues are dead and flaccid, and appear typically white, or sometimes greenish-, yellowish-, or grayish-

Fig. 10. Soybean seed with typical symptom of weathering injury.

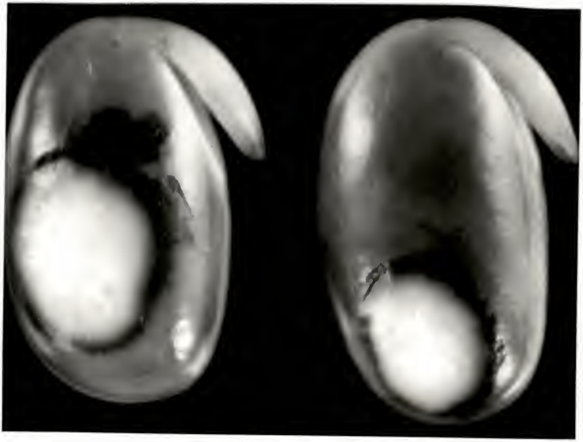


Fig. 11. Soybean seeds with typical symptoms of stink bug damage.

white. A distinct dark-red boundary commonly exists between damaged and sound tissues. Multiple lesions on a single seed might occur, and if they overlap, the typical circular wound will not be distinguishable. Frequently a minor puncture caused by the insect can be noticed in the center of the circular lesion. Deep punctures by the insect might result in inoculation of central seed tissues by N. coryli. Therefore, colonization of the tissues by the fungus will cause internal damage that is not always revealed on the outside of the seed.

Heat and drought damage are found in seeds of certain cultivars, when high temperatures (above 30°C) and drought occur during the seed-filling period. Visible symptoms on dry seeds are variable. Typical lesions may range from as little as a dimple on the cotyledonary area opposite the hilum, to completely shriveled and distorted seeds. Some seeds produced under these stressful conditions may become impermeable to water. When dimpled seeds are stained, dark-red or white patches occur in the lesioned tissues, and can be mistaken for weathering damage. The presence of dimples on dry seeds will guide the analyst to avoid misinterpretations. Shriveled seeds, after staining, might be deformed, and reveal white and dark-red patches scattered over the cotyledons, with higher concentration on the upper half of the seed, close to the embryonic axis (Fig. 12). Dead tissues are flaccid and friable. Severely shriveled seeds will not germinate due to a complete crushing of the tissues of the embryonic axis, and upper cotyledons.

Freeze injury in soybean seeds, as described by Moore (1973), will vary with the stage of development of the seed when exposed, temperature and duration of frost period. Immature seeds are generally killed, and

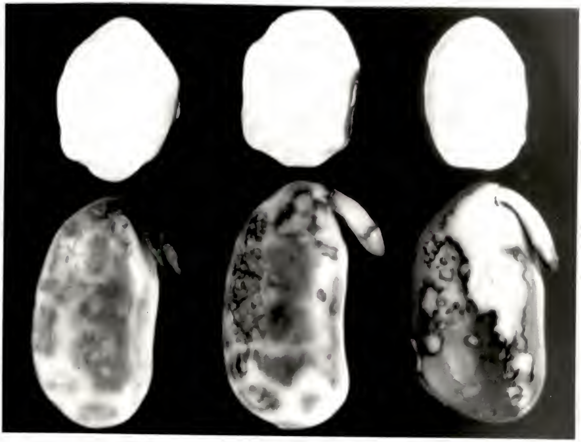


Fig. 12. Soybean seeds with lesions due to heat and drought stress. Top: dry seeds; bottom: stained seeds.

remain green; dry and mature seeds tend to resist damage. Damaged tissues are identified with the tz test by embryos being darker red than normal and by a tendency to release leachate that produces a red precipitate, which accumulates within seedcoats and in the testing solution. Freeze injured tissues, as revealed by Osorio (1987), tend to develop a greenish- or brownish-red appearance.

More than one of the above described defects can be observed on a single seed. Other damages are associated with ageing and poor storage conditions. Warm and humid conditions during storage will result in severely damaged seeds. With the tz test the symptoms of improper storage are similar to the symptoms for weathering. The correct diagnosis of the cause of seed deterioration can be achieved by conducting a bio-assay, such as the blotter test. High levels of infection by storage fungi, such as *Aspergillus* spp. and *Penicillium* spp., will be found on seeds with storage problems, while *Phomopsis* spp., *Fusarium* spp., *Cercospora kikuchii*, or other field pathogens will normally be found on seeds with weathering damage.

The identification of these causes for seed weaknesses might seem complex at the first reading, but with good training and experience the seed analyst can readily recognize and differentiate these symptoms.

Identification of Levels of Viability

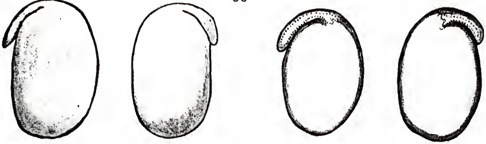
The tz test is based upon the analysis of the condition of individual seeds. Each seed is rated as viable or non-viable, and the causes for seed weaknesses are recorded. Moore and Smith, as cited by Copeland et al. (1959), and Moore (1961, 1962b, 1967) have worked out a relative classification for corn and soybean seed. Each seed was assigned a soundness rating of 1 to 5, if viable, and of 6 to 8, if non-viable. The presence, location and nature of staining, and the physical condition of embryo structures are used in this classification. This methodology was modified and described in detail for soybean seed by Franca Neto et al. (1985b). A brief description of each category of seed viability follows.

Categories 1 to 3: viable and vigorous seeds.

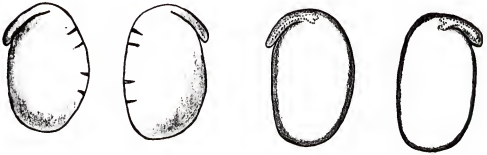
Category 1: all embryo structures are sound. Uniform and superficial staining indicates a slow penetration of the tz-solution. Internal surfaces of the cotyledons are stained only at the borders. All embryo tissues are normal and turgid (Fig. 13a).

Category 2: small and superficial damages are noted on the outer surfaces of the cotyledons; internal surfaces do not have any sign of damage. A category 2 seed with minor signs of weathering damage is shown in Fig. 13b.

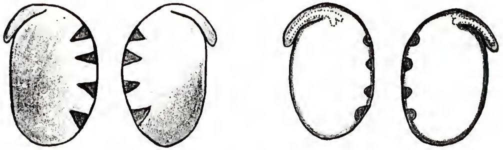
Category 3: superficial dark-red or white areas are present on outer surfaces of the cotyledons (Fig. 13c), or very superficial damage on the cortex of the hypocotyl/radicle axis, but not touching the stele (Fig. 13d). Internal surface of seed might present darker spots due to the



a: category 1



b: category 2



c: category 3

d: category 3

Fig. 13. Illustration of different levels of viability of soybean seeds: a) category 1; b) category 2; c) category 3, cotyledonary damage; d) category 3, damage to embryonic axis.

lesions, but not deeper than 0.5 mm. Seeds in these three categories are viable and vigorous, and they usually show a fast speed of germination and emergence.

Categories 4 and 5: viable, non-vigorous seeds.

Category 4: dark-red or white tissues are present on both cotyledons, with an affected surface of less than 50% of the cotyledons. Damage is visible on the inner surface of the cotyledons. The junction between the embryonic axis and cotyledons must be unaffected, and the stele as well (Fig. 14a).

Category 5: cotyledons are damaged severely but 50% or more of storage tissue is still viable and functional. Vascular region close to the point of attachment between embryonic axis and one cotyledon might be affected. Embryonic axis is still well defined and viable (Fig. 14b). Seeds ranked as category 5 will germinate and produce a normal seedling only under ideal conditions.

The critical area of the evaluation is between categories 5 and 6. If a sample contains many seeds rated as 5 or 6, there could be discrepancies between the results of the standard germination test and the tz test.

Categories 6 to 8: non-germinable seeds.

Category 6: this category is characterized by presence of similar lesions as the ones described for category 5. However, the amount of damaged tissue is larger, making the seed non-viable.

Category 7: deep damage to the stele is present (Fig. 14c). The vascular region between the embryonic axis and both cotyledons is

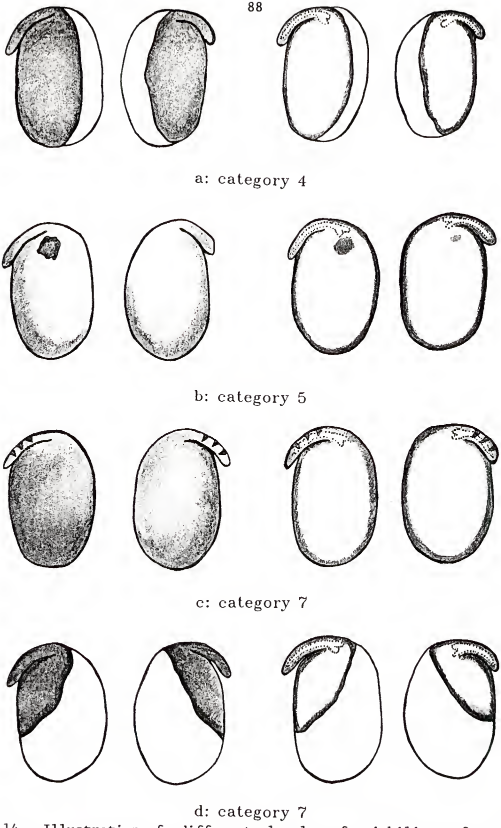


Fig. 14. Illustration of different levels of viability of soybean seeds: a) category 4; b) category 5; c) category 7, cotyledonary damage; d) category 7, damage to embryonic axis.

severely damaged. Damage to the plumule might be present. More than 50% of the reserve tissues are deteriorated (Fig. 14d).

Category 8: all embryo structures are dead, with flaccid and friable tissues.

Recording and Interpreting the Results

Each seed of a seed sample is graded into one category of viability, and the kind(s) of weakness on each seed is recorded, using a report form, as suggested by Franca Neto et al. (1985b). After 100 seeds (2 X 50 seeds) from a seed sample are evaluated, the percentages of seed rated within each category (1 to 8) are determined. The germination potential is calculated by the summation of the percentages of seeds in categories one through five. The vigor rating is determined by the total of seeds within categories one through three.

The vigor level can be interpreted according to this classification:

-very high: $\geq$ 80%;

-high: 70% to 79%;

-medium: 50% to 69%;

-low: 30% to 49%;

-very low: $\leq$ 29%.

The diagnosis for the cause(s) of low seed quality is achieved by the determination of the percentages of seeds affected by each kind of weakness, such as mechanical damage, weathering and stink bug injury, from categories 6 through 8. These levels of damage may be interpreted according to the following classification:

-no serious problem (acceptable): $\leq$ 6%;

-serious problem: 7% to 10%;

-very serious problem: > 10%.

If a serious or very serious problem is diagnosed by the seed analyst using the tz test, e.g., a high level of mechanical damage, or stink bug damage, a corrective action can be taken to improve seed quality. The seed producer can be advised, for example, to adjust the threshing system of his combine harvester, or to improve his methods for the control of stink bugs. Examples of results provided by the tz test are illustrated in Table 21. Seed lot no. 1 shows good germination and vigor, and no serious problems with mechanical damage, weathering, or stink bug damage. Seed lot no. 2 has germination near 80%, and the vigor level was rated as medium, mainly due to serious problems with mechanical damage. Lot no. 3 had 75% germination, and low (49%) vigor, due to both weathering and stink bug damage.

An example of the tremendous value of feedback to producers of information provided by the tz test is illustrated by Costa et al. (1987). High levels of mechanical damage were determined for soybean seeds produced in the state of Parana, Brazil. After the problem was identified, farmers were instructed how to improve the adjustments of the combine, through wide-scale extension training programs. In two years, levels of mechanical damage on soybean seed were drastically reduced to the acceptable levels of $\leq$ 6%.

Table 21. Illustration of the results provided by the tetrazolium test for three soybean seed lots.

Parameter	Lot #1	Lot #2	Lot #3
		%	
Germination	93	82	75
Vigor[a]	79	65	49
Mechanical damage	2[b]	10	5
Weathering	4[b]	5	12
Stink bug damage	1[b]	4	9

[a]Vigor level: very high: $\geq$ 80%; high: 70-79%; medium: 50-69%; low: 30-49%.

[b]Percentage of loss of germination due to a specific damage.

Advantages and Limitations of the Test

Some of the major positive and negative aspects of the tz test for evaluating soybean seed quality have been listed (Cottrell, 1948; Delouche et al., 1962; Franca Neto et al., 1985b; Grabe, 1959; Lakon, 1949; Moore, 1962b).

Advantages

Listed below are the major advantages of the tz test.

a) it by-passes major environmental disturbances that might affect the performance of growth tests;

b) focuses attention on the physical and physiological conditions of the embryo structures of each individual seed;

c) provides quick evaluation: 18 h for soybean;

d) allows identification of level of seed vigor;

e) diagnoses the causes of seed deterioration;

f) requires only simple and inexpensive equipment.

Limitations

The major disadvantages of the tz test are listed.

a) it requires knowledge of the seed structures and interpretation techniques;

b) is relatively tedious, because examination of individual seeds requires patience and experience;

c) consumes more time per sample than the standard germination test in spite of being a quick test; however, the tz test provides more information than the standard germination test.

Mason et al. (1982) reported that the tz test was not effective in detecting recently induced mechanical damage. This problem can be easily overcome with the use of lower concentrations (0.075%) of the tz-solution.

Precision and Accuracy of Results

A good level of reliability and precision of the tz test was demonstrated by Franca Neto et al. (1986). Several samples of soybean seed were sent to 41 seed laboratories, with specific instructions for the performance of the following tests: a) standard germination, as prescribed by the Rules for Testing Seeds (AOSA, 1981); b) accelerated aging by the tray and chamber methods, according to the procedures described in the Seed Vigor Testing Handbook (AOSA, 1983); c) tz test, as described by Franca Neto et al. (1985b); and d) emergence in sand, according to specific procedures sent with the samples. The tz test was ranked as the second most precise test with regards to repeatability, after the standard germination test.

In most situations, the percentage of germinable seed in the tz test and the standard germination test are similar. Up to 5.0% difference between these tests is considered acceptable. However, discrepancies might exist, and might be due to one of the following reasons: a) sampling differences; b) improper tz testing techniques; c) improper

techniques in the standard germination test; d) presence of hard seeds in the sample; e) use of seed lots with low or medium vigor; f) presence of seeds with high levels of mechanical or stink bug damages; g) presence of high levels of seed infected by *Phomopsis* spp., *Fusarium* spp. or *Colletotrichum truncatum*.

Lakon, as pointed out in a report by Gadd (1950, p. 253), stated that his "long experience in comparing the tetrazolium method and the ordinary germination methods has shown that where there are differences it was the germination test which failed." The occasional superior performance of the tz test over the standard germination test was also found for soybean seeds produced in Florida.

Research Data From Florida

Seventy-three samples of soybean seeds of 23 cultivars and five breeding lines produced in Florida in 1986 were evaluated for quality at the Agronomy Seed Laboratory of the University of Florida.

The tetrazolium test was performed according to the previously described procedures. In addition to the tz test, the following tests were done: standard germination (rolled paper toweling), emergence-in-sand, and blotter. The tests were performed on the seed samples according to procedures described in Chapter II. Several regression and simple correlation analyses were performed on the data set.

Results and Discussion

Ranges and means for several parameters of seed quality for the 73 samples of soybean seed are given in Table 22. A wide range of quality was found among the seed samples: standard germination varied from as low as 34% to as high as 96%. On the average, tz-vigor was medium (62.5%). Levels of mechanical damage, as revealed by categories 6 through 8 in the tz test, varied from 0% to 27%, and on the average these levels indicated very serious problems, with a mean value of 10.2% of non-germinable seeds due to mechanical damage. Weathering and stink bug damage showed a similar range of variation as for mechanical damage, and on the average were rated as serious, with mean values of 6.8% and 7.1% respectively.

Seed samples were arbitrarily grouped according to the incidence of seed infection by Phomopsis spp.: high, with incidence ranging from 33% to 77% (18 samples); medium, from 11% to 32% (27 samples); and low, from 0% to 10% (28 samples). Using the difference between standard and tz-germination as one variable and incidence of Phomopsis spp. as the other, simple correlations were performed within each level of incidence of Phomopsis spp. Highly significant correlation (0.75; $P \leq 0.001$) was observed within the samples with high incidence of Phomopsis spp., meaning that the higher the incidence of seed infection by Phomopsis spp., the higher the discrepancy between tz-germination and standard germination. One of the reasons for these discrepancies was explained in Chapter III: the higher the incidence of Phomopsis spp., the lower the germination by the standard (rolled-paper-towel) test. No

Table 22. Ranges and means for several quality parameters for 73 samples of soybean seeds.

Parameter	Range	Mean
	---------------%---------------	
Standard germination	34-96	70.3
Emergence in sand	43-99	75.3
Tz-germination	55-99	78.9
Tz-vigor	30-99	62.5
Tz-mechanical damage	0-27	10.2
Tz-weathering	0-28	6.8
Tz-stink bug	0-28	7.1
Damping-off	0-15	4.6
Phomopsis spp.	0-77	20.1

significant correlations were obtained for seed samples with medium and low levels of Phomopsis spp. Discrepancies larger than the acceptable level of 5% between tz-germination and standard germination occurred in 60% of the seed samples. There are several reasons for such discrepancies, as previously described in this Chapter, but they were particularly high for samples of soybean seed with high incidence of Phomopsis spp. Discrepancies (>5%) between the results of these two tests were observed on 83% of the samples with high incidence of Phomopsis spp. (mean discrepancy of 17%, maximum discrepancy of 49%).

As demonstrated in Chapters II and III, the standard germination test is not an accurate method to evaluate germination of soybean seeds with high levels of infection by Phomopsis spp. It was also demonstrated in those Chapters that in situations like this, emergence in sand or the tz test provided more reliable estimates of seed viability. When the results of the standard germination test and tz test are correlated to the ones obtained by the emergence-in-sand test for the 73 seed samples (Table 23), better correlations were obtained for tz-germination versus emergence in sand than for standard germination and emergence in sand. This tendency is also illustrated by the regression analyses contained in Figs. 15 and 16.

Another interesting result provided by the tz test was that stink bug damage may result in seedling damping-off in the emergence in sand test, as demonstrated by the significant ($P \leq 0.001$) correlation coefficient (0.36) between level of damping-off and amount of stink bug damage. The damping-off was probably due to the yeast fungus Nematospora coryli, which is inoculated into the seed by the stink bug.

Table 23. Correlations of standard germination and emergence in sand versus several seed quality parameters obtained by the tz test.

Parameter	Standard germination	Emergence in sand
Tz-germination	0.65[a]***	0.88***
Tz-vigor	0.66***	0.82***
Tz-mechanical damage	-0.49***	-0.63***
Tz-weathering	-0.48***	-0.65***
Tz-stink bug	-0.42***	-0.56***

[a]Correlation coefficient.
*** $P \leq 0.001$

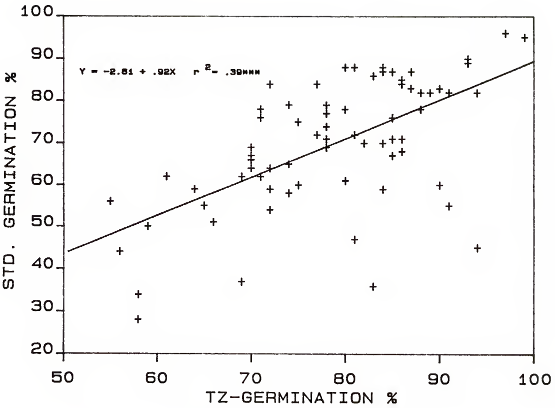


Fig. 15. Regression analysis for germination potential determined by the tz test versus standard germination for 73 samples of soybean seed.

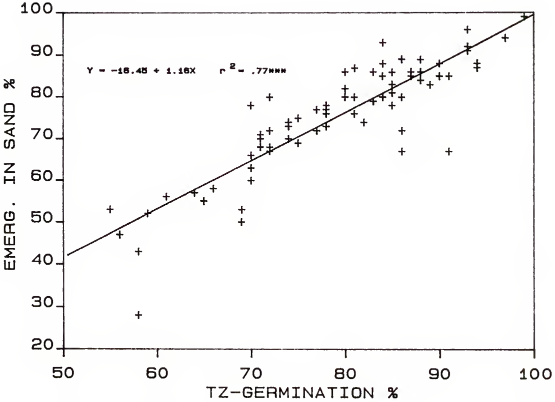


Fig. 16. Regression analysis for germination potential determined by the tz test versus emergence in sand for 73 samples of soybean seed.

Summary and Conclusions

Detailed procedures for the tz test were provided for soybean seed. In addition to determine estimates of vigor and germination potentials, the tz test can be used to diagnose the possible causes, such as mechanical damage, weathering, and stink bug injuries, that can contribute to lowering seed quality. The identification of the causes of seed weakness and its feedback to seed producers will enable the producers to make corrections to promote better soybean seed quality in future crops.

The tz test is a useful tool in production and quality research of soybean seed. The reliability of the test can be improved by the use of specific and detailed guidelines, by the standardization of the test procedures, and by training programs for seed analysts. Complementary bio-assays are sometimes needed in order to more precisely identify the causes of poor seed quality.

Finally, as stated by Moore (1969, p. 239), "anyone attempting to study seed life would do well to take time out to gain a 'tetrazolium viewpoint' of characteristics that make seeds weak or non-germinative. With this knowledge and that of major historical events supporting tetrazolium seed testing, one can be guided around pitfalls that hinder testing programs."

CHAPTER VII
SUMMARY AND CONCLUSIONS

There are several factors that contribute to lowering the quality of soybean seed. The major ones are mechanical and weathering damages, stink bug damage, heat and drought damage, and freeze injury. Several pathogens also affect soybean seed quality. Phomopsis spp., Colletotrichum truncatum (Schw.) Andrus and Moore, Cercospora kikuchii Matsu and Tomoyasu, and Fusarium spp. are among the fungi most frequently associated with soybean seeds (Henning, 1985). In spite of being distinct factors, the action and interaction of all these physiological, physical, and pathological factors contribute to a common result: seed deterioration.

Several experiments were conducted to study how the above factors can influence the quality of soybean seeds. The studies reported in Chapter II were conducted with the objectives: a) to evaluate the physiological and pathological qualities of commercial soybean seeds produced in Florida; and b) to diagnose the major factors that decrease soybean seed quality and recommend practices that might result in improvement of quality of soybean seeds produced in Florida. The quality of soybean seeds produced in Florida is affected by several different types of damage, incidence of specific fungi, cultivar, and location of production. Mechanical damage during harvest was the most

detrimental factor affecting quality of soybean seeds, followed by weathering and then stink bug damage. Phomopsis spp. and Fusarium spp. were the fungi most frequently associated with soybean seed produced in Florida in 1986. Kirby, a late maturity cultivar, maintained the best seed quality when compared to Centennial and Gordon. Due to its climatic conditions, seed produced in the eastern region of the Florida panhandle (Bascom, Marianna, Graceville, and Greenwood) had lower levels of infection by several fungi. Apparently, this region is more suitable than other regions in Florida for production of soybean seeds. There was evidence that rolled paper toweling is not the best substrate for evaluating viability of soybean seed lots with high levels of Phomopsis spp. Better crop management practices would improve the quality of commercial soybean seed produced in Florida. Better crop management includes more timely harvest, better adjustments of combine harvesters, better control of stink bugs, and selection of more appropriate regions for seed production.

Chapter III reports on research conducted with the objectives: a) to determine the influence of Phomopsis spp. in evaluating germination of soybean seeds by the standard germination, emergence-in-sand, and tetrazolium tests; and b) to recommend alternative methods that correctly evaluate germination of soybean seed lots infected with Phomopsis spp. The rolled-paper-towel test was not an accurate method to evaluate germination of soybean seed lots with high incidence of Phomopsis spp. The estimates of viability provided by this test were biased by the action of Phomopsis spp. from soybean seeds with high incidence of this fungus. The tz test, being a biochemical test, was

not influenced by the presence of fungi within the soybean seedcoat. Emergence in sand was not influenced by seed infection by Phomopsis spp., if seed vigor, as expressed by tz test, was high. As the vigor determined by the tz test decreased, the rate of germination and emergence as expressed by the emergence in sand test also decreased. Low-vigor seed samples were more vulnerable to the action of fungi in the seedcoats, thus resulting in higher levels of seedling infection and damping-off. The emergence-in-sand test provided estimates of germination for seed lots with high incidence of infection by Phomopsis spp. that more nearly simulated what would be expected in the field under ideal conditions. The results of germination from the tz test were similar to the results from the emergence-in-sand test. Therefore, the tz test also provided unbiased estimates of viability for seed lots with high incidence of seed infection by Phomopsis spp. Standardization of procedures for the emergence test in sand or in a soil substitute is needed to improve reliability and to decrease variation in results among laboratories, as reported by Franca Neto et al. (1986) and Skinner and Schroeder (1978).

The objectives of the study reported in Chapter IV were: a) to determine the influence of Colletotrichum truncatum on evaluating the viability of soybean seeds by the standard germination, emergence in sand, and tetrazolium tests; b) to determine the best method to evaluate germination of seeds infected with the pathogen; and c) to investigate the depth of infection by this pathogen in soybean seeds. Soybean seed infection by C. truncatum was mainly confined to the seedcoat. However, embryo infection was not rare. Additionally, seed infection by this

pathogen was a major source of seedling damping-off, regardless of seed vigor. The detection of seed viability by the tz test was not influenced by the amount of seed infected by C. truncatum. Due to the possibility of occurrence of damping-off, if seeds are planted, the tz test would overestimate the viability of soybean seed lots infected with high incidence of this pathogen. In contrast, the rolled paper toweling method underestimated viability for seed lots with high incidence of this fungus. Emergence in sand provided more reliable estimates of viability of seed lots infected with C. truncatum. The conclusions in this chapter were based on analyses performed on seed samples that were not treated with any fungicide. Additionally, this experiment was not designed to study the dissemination of the pathogen or the control of the disease. Further investigations are necessary to determine the effects of fungicide treatment of soybean seed infected with C. truncatum on seed quality, and on control and spread of the pathogen.

The main objective of the study in Chapter V was to determine the effects of Cercospora kikuchii on the quality of soybean seeds produced in Florida. In addition, a discussion of possible reasons for some inconsistent results and conclusions reported in the literature about seed infection of soybean seeds by C. kikuchii were presented. Soybean seed infection by C. kikuchii was almost exclusively confined to the seedcoat. An antagonistic effect was observed between C. kikuchii and Phomopsis spp. infecting soybean seeds: the higher the level of seed infection by C. kikuchii, the lower the seed infection by Phomopsis spp. No detrimental effects of seed infection of this pathogen were observed on germination, emergence, and vigor of soybean seeds with the natural

field incidence of purple stained seeds used in this study. Inconsistent conclusions obtained by various researchers apparently were associated with different methodological approaches. Detrimental effects of seed infection by C. kikuchii on the quality of soybean seeds were reported by those researchers who provided maximum opportunity for a detrimental effect, i.e., 100% purple stained seeds were compared to symptomless seeds. Whereas, researchers who used normal field infection levels of C. kikuchii found no significant effect of seed infection by the pathogen on the quality of soybean seed.

The following information on tetrazolium testing of soybean seeds is considered in Chapter VI: a) the major events and accomplishments which contributed to the development and perfecting of the test; b) the basic principles of the test; c) needed equipment and supplies; d) procedures for seed preparation and evaluation; e) basis for the correct interpretation of the results; f) advantages and limitations of the test; and g) accuracy of the results. Detailed procedures for the tz test were provided for soybean seed. In addition to determining estimates of vigor and germination potentials, the tz test can be used to diagnose the possible causes, such as mechanical damage, weathering, and stink bug injury, that can contribute to reduced seed quality. The identification of the causes of seed weakness and its feedback to seed producers will enable producers to make corrections to promote better soybean seed quality in future crops. The tz test is a useful tool in production and quality research of soybean seed. The reliability of the test can be improved by the use of specific and detailed guidelines, by the standardization of the test procedures, and by training programs for

seed analysts. Complementary bio-assays are sometimes needed in order to more precisely identify the causes of poor seed quality. Finally, as stated by Moore (1969, p. 239), "anyone attempting to study seed life would do well to take time out to gain a 'tetrazolium viewpoint' of characteristics that make seeds weak or non-germinative. With this knowledge and that of major historical events supporting tetrazolium seed testing, one can be guided around pitfalls that hinder testing programs."

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BIOGRAPHICAL SKETCH

José de Barros França Neto was born on February 07, 1954, in Sao Paulo, State of Sao Paulo, Brazil. He completed high school at the "Colegio Rio Branco" in Sao Paulo, State of Sao Paulo, in December, 1971. In 1975, he received his Bachelor of Science degree in Agronomy from the University of Sao Paulo, "Escola Superior de Agricultura Luiz de Queiroz", in Piracicaba, State of Sao Paulo. From August, 1975 to April, 1976, he worked at the Souza Dias Seed Company, in Assis, Sao Paulo. From May to September, 1976, he attended the Seed Improvement Course at Mississippi State University. From January 1977 to December 1978 he worked at Mississippi State University towards a Master of Science degree in Agronomy, Seed Technology.

In 1979 he joined the seed technology staff of the National Center for Soybean Research of the Brazilian Corporation for Agricultural Research (EMBRAPA), located at Londrina, State of Parana. From 1979 to 1985 he conducted research on seed quality of soybean and sunflower.

In 1986, he was granted a scholarship from EMBRAPA to pursue a Ph.D. degree in Agronomy at the University of Florida. He is currently a Ph.D. candidate, and upon completion of his graduate work, he will return to the Soybean Center, where he will continue to conduct research on soybean seed quality.

On April 20, 1989 he received the Fred G. Hull Agronomy Research and Achievement Award from the Institute of Food and Agricultural Sciences and the Department of Agronomy, University of Florida. This prize was awarded in recognition of his outstanding research and his leadership and service in the field of agronomy.

He is a member of Gamma Sigma Delta, the American Society of Agronomy, Crop Science Society of America, Association of Official Seed Analysts, Soil and Crop Science Society of Florida, and the Brazilian Association of Seed Technologists.

He is married to Maria Rosane and has three children: Andrea, Marcelo, and Luciana.

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

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